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<p>(21) International Application Number: PCT/US00/07946 (22) International Filing Date: 24 March 2000 (24.03.00) (30) Priority Data: 60/126,603 25 March 1999 (25.03.99) US (63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application US 60/126,603 (CIP) Filed on 25 March 1999 (25.03.99) (71) Applicants (for all designated States except US): BASF AKTIENGESSELLSCHAFT [DE/DE]; Carl-Bosch Strasse 38, D-67056 Ludwigshafen (DE). GENETICS INSTITUTE INC. [US/US]; 87 CambridgePark Drive, Cambridge, MA 02140 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): SALFELD, Jochen, G. [DE/US]; 177 Old Westborough Road, North Grafton, MA 01536 (US). ROGUSKA, Michael [US/US]; 16 Hilldale Road, Ashland, MA 01721 (US). PASKIND, Michael [US/US]; 253 Redemption Rock Trail, Sterling,</p>		<p>MA 01564 (US). BANERJEE, Subhashis [IN/US]; 25 Hapgood Way, Shrewsbury, MA 01545 (US). TRACEY, Daniel, E. [US/US]; 149 Shaker Road, Harvard, MA 01451 (US). WHITE, Michael [US/US]; 30 Angelica Drive, Framingham, MA 01701 (US). KAYMAKALAN, Zehra [TR/US]; 4 Picadilly Way, Westborough, MA 01581 (US). LABKOVSKY, Boris [US/US]; 107A-5 Broadmeadow Road, Marlborough, MA 01752 (US). SAKORAFAS, Paul [US/US]; 47 Court Street, Newton, MA 02160 (US). FRIEDRICH, Stuart [CA/US]; 20 Albion Street, Melrose, MA 02176 (US). MYLES, Angela [US/US]; Apartment #7, 11 Crescent Drive, Andover, MA 01810 (US). VELDMAN, Geertruida, M. [NL/US]; 60 Woodmere Drive, Sudbury, MA 01776 (US). VENTURINI, Amy [US/US]; 207 Woburn Street, Lexington, MA 02420 (US). WARNE, Nicholas, W. [US/US]; 27 Fairwood Drive, Andover, MA 01810 (US). WIDOM, Angela [US/US]; 19 Cherokee Road, Acton, MA 01720 (US). ELVIN, John, G. [GB/GB]; 36 Perowne Street, Cambridge CB1 2AY (GB). DUNCAN, Alexander, R. [GB/GB]; 3 Hauwxtan Road, Little Shelford, Cambridge CB2 5HJ (GB). DERBYSHIRE, Elaine, J. [GB/GB]; 15 The Brambles, Studlands Rise, Royston, Hertfordshire SG8 9NQ (GB). CARMEN, Sara [GB/GB]; 136 Sturton Street, Cambridge CB1 2QF (GB). SMITH, Stephen [GB/GB]; 33 Church Road, Wicken, Ely, Cambridgeshire CB7 5XT (GB). HOLTET, Thor, Las [DK/GB]; 13 Mortimer Road, Royston, Hertfordshire (GB). DU FOU, Sarah, L. [GB/GB]; 76 Ickleford Road, Hitchin, Hertfordshire SG5 1TL (GB). (74) Agents: KARA, Catherine, J. et al.; Lahive &amp; Cockfield, LLP, 28 State Street, Boston, MA 02109 (US). (81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW. ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</p>
<p>(54) Title: HUMAN ANTIBODIES THAT BIND HUMAN IL-12 AND METHODS FOR PRODUCING (57) Abstract Human antibodies, preferably recombinant human antibodies, that specifically bind to human interleukin-12 (hIL-12) are disclosed. Preferred antibodies have high affinity for hIL-12 and neutralize hIL-12 activity <i>in vitro</i> and <i>in vivo</i>. An antibody of the invention can be a full-length antibody or an antigen-binding portion thereof. The antibodies, or antibody portions, of the invention are useful for detecting hIL-12 and for inhibiting hIL-12 activity, e.g., in a human subject suffering from a disorder in which hIL-12 activity is detrimental. Nucleic acids, vectors and host cells for expressing the recombinant human antibodies of the invention, and methods of synthesizing the recombinant human antibodies, are also encompassed by the invention.</p>		

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Consistent with the preponderance of Th1 responses in autoimmune diseases and the proinflammatory activities of IFN $\gamma$ , IL-12 may play a major role in the pathology associated with many autoimmune and inflammatory diseases such as rheumatoid arthritis (RA), multiple sclerosis (MS), and Crohn's disease.

5 Human patients with MS have demonstrated an increase in IL-12 expression as documented by p40 mRNA levels in acute MS plaques. (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996). In addition, *ex vivo* stimulation of antigen-presenting cells with CD40L-expressing T cells from MS patients resulted in increased IL-12 production compared with control T cells, consistent with the observation that CD40/CD40L  
10 interactions are potent inducers of IL-12.

Elevated levels of IL-12 p70 have been detected in the synovia of RA patients compared with healthy controls (Morita *et al* (1998) *Arthritis and Rheumatism.* 41: 306-314). Cytokine messenger ribonucleic acid (mRNA) expression profile in the RA synovia identified predominantly Th1 cytokines. (Bucht *et al.*, (1996) *Clin. Exp.*  
15 *Immunol.* 103: 347-367). IL-12 also appears to play a critical role in the pathology associated with Crohn's disease (CD). Increased expression of IFN $\gamma$  and IL-12 has been observed in the intestinal mucosa of patients with this disease (Fais *et al.* (1994) *J. Interferon Res.* 14:235-238; Parronchi *et al.*, (1997) *Am. J. Path.* 150:823-832; Monteleone *et al.*, (1997) *Gastroenterology.* 112:1169-1178, and Berrebi *et al.*, (1998)  
20 *Am. J. Path.* 152:667-672). The cytokine secretion profile of T cells from the lamina propria of CD patients is characteristic of a predominantly Th1 response, including greatly elevated IFN $\gamma$  levels (Fuss, *et al.*, (1996) *J. Immunol.* 157:1261-1270). Moreover, colon tissue sections from CD patients show an abundance of IL-12 expressing macrophages and IFN $\gamma$  expressing T cells (Parronchi *et al* (1997) *Am. J.*  
25 *Path.* 150:823-832).

Due to the role of human IL-12 in a variety of human disorders, therapeutic strategies have been designed to inhibit or counteract IL-12 activity. In particular, antibodies that bind to, and neutralize, IL-12 have been sought as a means to inhibit IL-12 activity. Some of the earliest antibodies were murine monoclonal antibodies (mAbs),  
30 secreted by hybridomas prepared from lymphocytes of mice immunized with IL-12 (see *e.g.*, World Patent Application Publication No. WO 97/15327 by Strober *et al.*; Neurath *et al.* (1995) *J. Exp. Med.* 182:1281-1290; Duchmann *et al.* (1996) *J. Immunol.* 26:934-

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938). These murine IL-12 antibodies are limited for their use *in vivo* due to problems associated with administration of mouse antibodies to humans, such as short serum half life, an inability to trigger certain human effector functions and elicitation of an unwanted immune response against the mouse antibody in a human (the "human anti-mouse antibody" (HAMA) reaction).

In general, attempts to overcome the problems associated with use of fully-murine antibodies in humans, have involved genetically engineering the antibodies to be more "human-like." For example, chimeric antibodies, in which the variable regions of the antibody chains are murine-derived and the constant regions of the antibody chains are human-derived, have been prepared (Junghans, *et al.* (1990) *Cancer Res.* 50:1495-1502; Brown *et al.* (1991) *Proc. Natl. Acad. Sci.* 88:2663-2667; Kettleborough *et al.* (1991) *Protein Engineering.* 4:773-783). However, because these chimeric and humanized antibodies still retain some murine sequences, they still may elicit an unwanted immune reaction, the human anti-chimeric antibody (HACA) reaction, especially when administered for prolonged periods.

A preferred IL-12 inhibitory agent to murine antibodies or derivatives thereof (*e.g.*, chimeric or humanized antibodies) would be an entirely human anti-IL-12 antibody, since such an agent should not elicit the HAMA reaction, even if used for prolonged periods. However, such antibodies have not been described in the art and, therefore are still needed.

### Summary of the Invention

The present invention provides human antibodies that bind human IL-12. The invention also relates to the treatment or prevention of acute or chronic diseases or conditions whose pathology involves IL-12, using the human anti-IL-12 antibodies of the invention.

In one aspect, the invention provides an isolated human antibody, or an antigen-binding portion thereof, that binds to human IL-12.

In one embodiment, the invention provides a selectively mutated human IL-12 antibody, comprising:



a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue such that it binds to human IL-12.

In a preferred embodiment, the invention provides a selectively mutated human  
5 IL-12 antibody, comprising:

a human antibody or antigen-binding portion thereof, selectively mutated at a preferred selective mutagenesis position with an activity enhancing amino acid residue such that it binds to human IL-12.

In another preferred embodiment, the selectively mutated human IL-12 antibody  
10 or antigen-binding portion thereof is selectively mutated at more than one preferred selective mutagenesis position, contact or hypermutation positions with an activity enhancing amino acid residue. In another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof is selectively mutated at no more than three preferred selective mutagenesis positions, contact or hypermutation  
15 positions. In another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof is selectively mutated at no more than two preferred selective mutagenesis position, contact or hypermutation positions. In yet another preferred embodiment, the selectively mutated human IL-12 antibody or antigen-binding portion thereof, is selectively mutated such that a target specificity  
20 affinity level is attained, the target level being improved over that attainable when selecting for an antibody against the same antigen using phage display technology. In another preferred embodiment, the selectively mutated human IL-12 antibody further retains at least one desirable property or characteristic, *e.g.*, preservation of non-cross reactivity with other proteins or human tissues, preservation of epitope recognition,  
25 production of an antibody with a close to a germline immunoglobulin sequence.

In another embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a  $K_{off}$  rate constant of  $0.1 \text{ s}^{-1}$  or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro*  
30 phytohemagglutinin blast proliferation assay (PHA assay) with an  $IC_{50}$  of  $1 \times 10^{-6} \text{ M}$  or less. More preferably, the isolated human antibody or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-2} \text{ s}^{-1}$  or less, or

inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-7}$  M or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-3} s^{-1}$  or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-8}$  M or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-4} s^{-1}$  or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less. More preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-5} s^{-1}$  or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-10}$  M or less. Even more preferably, the isolated human antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-5} s^{-1}$  or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-11}$  M or less.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-6}$  M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6. In a preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 7; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 8.

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In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) . inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less;
- 5        b)        has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and
- c)        has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

In a preferred embodiment, the isolated human antibody, or an antigen-binding  
10        portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11; and has a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and has a light chain CDR1 comprising the amino acid sequence of SEQ ID  
15        NO: 14. In a preferred embodiment, the isolated human antibody has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15; and has a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 20        a)        . inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less;
- b)        has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and
- c)        has a light chain CDR3 comprising the amino acid sequence of SEQ ID  
25        NO: 18.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20. In a preferred embodiment, the isolated human antibody, or an antigen-binding  
30        portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22. In a preferred embodiment, the isolated human antibody, or an antigen-binding

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portion thereof, has the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant region selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions or any allelic variation thereof as discussed in Kabat *et al.* (Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), included herein by reference. In a more preferred embodiment, the antibody heavy chain constant region is IgG1. In another preferred embodiment, the isolated human antibody is a Fab fragment, or a F(ab')<sub>2</sub> fragment or a single chain Fv fragment.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC<sub>50</sub> of  $1 \times 10^{-9}$  M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; and
- c) has a light chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 335-SEQ ID NO: 403; and a light chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; and a light chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 470-SEQ ID NO: 505. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant

region, or an Fab fragment or a F(ab')<sub>2</sub> fragment or a single chain Fv fragment as described above.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 5 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC<sub>50</sub> of 1 x 10<sup>-9</sup> M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID  
10 NO: 26.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27; and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28. In a preferred embodiment, the isolated human antibody, or an antigen-binding  
15 portion thereof, has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30. In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, which has a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31, and a light chain variable region comprising the amino  
20 acid sequence of SEQ ID NO: 32. In a preferred embodiment, the isolated human antibody comprises a heavy chain constant region, or an Fab fragment, or a F(ab')<sub>2</sub> fragment or a single chain Fv fragment as described above.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 25 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC<sub>50</sub> of 1 x 10<sup>-6</sup> M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5,  
30 or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a k<sub>off</sub> rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid

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sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 5; and

- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a  $k_{off}$  rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 6.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13, or a mutant thereof having one or more amino acid substitutions at a contact position or a hypermutation position, wherein said mutant has a  $k_{off}$  rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 11, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 13; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a  $k_{off}$  rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10, a light chain

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CDR2 comprising the amino acid sequence of SEQ ID NO: 12, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- 5 a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less;
- b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO:  
10 21, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a  $k_{off}$  rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19, and a heavy chain  
15 CDR1 comprising the amino acid sequence of SEQ ID NO: 21; and
- c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22, or a mutant thereof having one or more amino acid substitutions at preferred  
20 selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a  $k_{off}$  rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.

- 25 The invention also provides nucleic acid molecules encoding antibodies, or antigen binding portions thereof, of the invention. A preferred isolated nucleic acid encodes the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17. The isolated nucleic acid encoding an antibody heavy chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody heavy chain  
30 variable region comprising the amino acid sequence of SEQ ID NO: 19. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 21. In another

embodiment, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23. In another embodiment, the isolated nucleic acid encodes the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18. The isolated nucleic acid encoding an antibody light chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 20. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 22. In another embodiment, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less;
- 15 b) comprises a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27 and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a  $k_{off}$  rate no more than 10-fold higher than the antibody comprising a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 27, and a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 29; and
- 20 c) comprises a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30, or a mutant thereof having one or more amino acid substitutions at a preferred selective mutagenesis position, contact position or a hypermutation position, wherein said mutant has a  $k_{off}$  rate no more than 10-fold higher than the antibody comprising a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 28, and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 30.



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A preferred isolated nucleic acid encodes the heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25. The isolated nucleic acid encoding an antibody heavy chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 27. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 29. In another embodiment, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31. In another embodiment, the isolated nucleic acid encodes the light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26. The isolated nucleic acid encoding an antibody light chain variable region. In another embodiment, the isolated nucleic acid encodes the CDR2 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 28. In another embodiment, the isolated nucleic acid encodes the CDR1 of the antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 30. In another embodiment, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 32.

In another aspect, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a  $k_{\text{off}}$  rate constant of  $0.1 \text{ s}^{-1}$  or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an  $\text{IC}_{50}$  of  $1 \times 10^{-6} \text{ M}$  or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the  $V_{\text{H}}3$  germline family, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from a member of the  $V_{\text{L}}1$  germline family, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact position or hypermutation position with an activity enhancing amino acid residue.

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In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a  $k_{off}$  rate constant of  $0.1\text{ s}^{-1}$  or less, as determined by surface plasmon resonance, or which  
5 inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an  $IC_{50}$  of  $1 \times 10^{-6}\text{ M}$  or less.

b) has a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 595-667, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact  
10 position or hypermutation position with an activity enhancing amino acid residue.

c) has a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 669-675, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

15 In another embodiment, the invention provides an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

a) that binds to human IL-12 and dissociates from human IL-12 with a  $k_{off}$  rate constant of  $0.1\text{ s}^{-1}$  or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast  
20 proliferation assay (PHA assay) with an  $IC_{50}$  of  $1 \times 10^{-6}\text{ M}$  or less.

b) has a heavy chain variable region comprising the COS-3 germline amino acid sequence, wherein the heavy chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

25 c) has a light chain variable region comprising the DPL8 germline amino acid sequence, wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

In another embodiment, the invention provides an isolated human antibody, or an  
30 antigen-binding portion thereof, which has the following characteristics:

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a) that binds to human IL-12 and dissociates from human IL-12 with a  $k_{off}$  rate constant of  $0.1 \text{ s}^{-1}$  or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an  $IC_{50}$  of  $1 \times 10^{-6} \text{ M}$  or less.

5           b) has a heavy chain variable region comprising an amino acid sequence selected from a member of the  $V_H3$  germline family, wherein the heavy chain variable region comprises a CDR2 that is structurally similar to CDR2s from other  $V_H3$  germline family members, and a CDR1 that is structurally similar to CDR1s from other  $V_H3$  germline family members, and wherein the heavy chain variable region has a mutation at  
10 a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue;

          c) has a light chain variable region comprising an amino acid sequence selected from a member of the  $V_L1$  germline family, wherein the light chain variable region comprises a CDR2 that is structurally similar to CDR2s from other  $V_L1$  germline  
15 family members, and a CDR1 that is structurally similar to CDR1s from other  $V_L1$  germline family members, and wherein the light chain variable region has a mutation at a preferred selective mutagenesis position, contact or hypermutation position with an activity enhancing amino acid residue.

          In a preferred embodiment, the isolated human antibody, or antigen binding  
20 portion thereof, has a mutation in the heavy chain CDR3. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the light chain CDR3. In another embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the heavy chain CDR2. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof,  
25 has a mutation in the light chain CDR2. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the heavy chain CDR1. In another preferred embodiment, the isolated human antibody, or antigen binding portion thereof, has a mutation in the light chain CDR1.

          In another aspect, the invention provides recombinant expression vectors  
30 carrying the antibody-encoding nucleic acids of the invention, and host cells into which such vectors have been introduced, are also encompassed by the invention, as are

methods of making the antibodies of the invention by culturing the host cells of the invention.

In another aspect, the invention provides an isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human IL-12, and at least one  
5 additional primate IL-12 selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12, cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the mouse IL-12.

In another aspect, the invention provides a pharmaceutical composition comprising the antibody or an antigen binding portion thereof, of the invention and a  
10 pharmaceutically acceptable carrier.

In another aspect, the invention provides a composition comprising the antibody or an antigen binding portion thereof, and an additional agent, for example, a therapeutic agent.

In another aspect, the invention provides a method for inhibiting human IL-12  
15 activity comprising contacting human IL-12 with the antibody of the invention, *e.g.*, J695, such that human IL-12 activity is inhibited.

In another aspect, the invention provides a method for inhibiting human IL-12 activity in a human subject suffering from a disorder in which IL-12 activity is detrimental, comprising administering to the human subject the antibody of the  
20 invention, *e.g.*, J695, such that human IL-12 activity in the human subject is inhibited. The disorder can be, for example, Crohn's disease, multiple sclerosis or rheumatoid arthritis.

In another aspect, the invention features a method for improving the activity of an antibody, or an antigen binding portion thereof, to attain a predetermined target  
25 activity, comprising:

- a) providing a parent antibody a antigen-binding portion thereof;
- b) selecting a preferred selective mutagenesis position selected from group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.
- 30 c) individually mutating the selected preferred selective mutagenesis position to at least two other amino acid residues to hereby create a first panel of mutated antibodies, or antigen binding portions thereof;

d) evaluating the activity of the first panel of mutated antibodies, or antigen binding portions thereof to determine if mutation of a single selective mutagenesis position produces an antibody or antigen binding portion thereof with the predetermined target activity or a partial target activity;

5 e) combining in a stepwise fashion, in the parent antibody, or antigen binding portion thereof, individual mutations shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof.

f) evaluating the activity of the combination antibodies, or antigen binding portions thereof to determine if the combination antibodies, or antigen binding portions  
10 thereof have the predetermined target activity or a partial target activity.

g) if steps d) or f) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial activity, additional amino acid residues selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A and L96 are mutated to at least two other amino  
15 acid residues to thereby create a second panel of mutated antibodies or antigen-binding portions thereof;

h) evaluating the activity of the second panel of mutated antibodies or antigen binding portions thereof, to determine if mutation of a single amino acid residue selected from the group consisting of H35, H50, H53, H54, H95, H96, H97, H98, L30A  
20 and L96 results in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

i) combining in stepwise fashion in the parent antibody, or antigen-binding portion thereof, individual mutations of step g) shown to have an improved activity, to form combination antibodies, or antigen binding portions thereof;

25 j) evaluating the activity of the combination antibodies or antigen binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity or a partial target activity;

k) if steps h) or j) do not result in an antibody or antigen binding portion thereof having the predetermined target activity, or result in an antibody with only a partial  
30 activity, additional amino acid residues selected from the group consisting of H33B, H52B and L31A are mutated to at least two other amino acid residues to thereby create a third panel of mutated antibodies or antigen binding portions thereof;

l) evaluating the activity of the third panel of mutated antibodies or antigen binding portions thereof, to determine if a mutation of a single amino acid residue selected from the group consisting of H33B, H52B and L31A resulted in an antibody or antigen binding portion thereof, having the predetermined target activity or a partial activity;

m) combining in a stepwise fashion in the parent antibody, or antigen binding portion thereof, individual mutation of step k) shown to have an improved activity, to form combination antibodies, or antigen binding portions, thereof;

n) evaluating the activity of the combination antibodies or antigen-binding portions thereof, to determine if the combination antibodies, or antigen binding portions thereof have the predetermined target activity to thereby produce an antibody or antigen binding portion thereof with a predetermined target activity.

In another aspect, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other contact or hypermutation position;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

5 In one embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity is not further improved by mutagenesis in said phage-display system;

10 b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby  
15 create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof;

e) repeating steps b) through d) for at least one other contact or hypermutation  
20 position;

f) combining, in the parent antibody, or antigen-binding portion thereof, individual mutations shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding  
25 portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58,  
30 H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32,

L53 and L93. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94.

In a more preferred embodiment, the contact positions are selected from the group  
5 consisting of L50 and L94.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof ;  
b) selecting a preferred selective mutagenesis position, contact or hypermutation  
10 position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expressing  
15 said panel in an appropriate expression system;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions  
20 thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristics, wherein the property or characteristic is one that needs to be retained in the antibody;

until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-  
25 binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-  
30 crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce



an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment of the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position, contact or hypermutation position;

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c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one  
10 other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) repeating steps a) through e) for at least one other preferred selective  
15 mutagenesis position, contact or hypermutation position;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

20 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

25 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope  
30 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred

embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting a contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected contact or hypermutation position;
- c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

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d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one other property or characteristics, wherein the property or characteristic is one that needs to be retained;

until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-

crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30,

H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline

immunoglobulin sequence. In a more preferred embodiment the residues for selective mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope

recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred

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embodiment, the contact positions are selected from the group consisting of L50 and L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer  
5 preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a recombinant parent antibody or antigen-binding portion thereof;  
10 that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting a preferred selective mutagenesis position, contact or hypermutation position within a complementarity determining region (CDR) for mutation, thereby identifying a selected preferred selective mutagenesis position contact or hypermutation  
15 position;

c) individually mutating said selected preferred selective mutagenesis position, contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

20 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residuc;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof for at least one  
25 other property or characteristic, wherein the property or characteristic is one that needs to be retained, until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

f) repeating steps a) through e) for at least one other preferred selective  
30 mutagenesis position, contact or hypermutation position;

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g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and at least one retained other characteristic, to form combination antibodies, or antigen-binding portions thereof; and

5 h) evaluating the activity of the combination antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

10 In a preferred embodiment, the contact positions are selected from the group consisting of H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope  
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In another preferred embodiment, the hypermutation positions are selected from the group consisting of H30, H31, H31B, H32, H52, H56, H58, L30, L31, L32, L53 and L93 and the other  
20 characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment the residues for selective  
25 mutagenesis are selected from the preferred selective mutagenesis positions from the group consisting of H30, H31, H31B, H32, H33, H52, H56, H58, L30, L31, L32, L50, L91, L92, L93, L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope  
30 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence. In a more preferred embodiment, the contact positions are selected from the group consisting of L50 and

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L94 and the other characteristic is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody  
5 with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;
  - b) selecting an amino acid residue within a complementarity determining region  
10 (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
  - c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions  
15 thereof;
  - d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;
  - e) evaluating the panel of mutated antibodies, or antigen-binding portions  
20 thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic;
- until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of  
25 non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

In another embodiment, the invention provides a method for improving the  
30 activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

5 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

10 e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

15 f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

25 a) providing a recombinant parent antibody or antigen-binding portion thereof; that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and;

30



c) individually mutating said selected contact or hypermutation position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof, and expressing said panel in a non-phage display system;

5 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in  
10 at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope  
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

20 a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A,  
25 H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

30 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) repeating steps b) through d) for at least one other position within the CDR which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 ;

5 f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity, to form combination antibodies, or antigen-binding portions thereof; and

g) evaluating the activity and other property or characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid  
10 residues, relative to the parent antibody or antigen-binding portion thereof; until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope  
15 recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

20 a) providing a parent antibody or antigen-binding portion thereof;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

25 c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof,  
30 thereby identifying an activity enhancing amino acid residue;

- 30 -

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof, relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

f) repeating steps b) through e) for at least one other CDR position which is  
5 neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity  
10 and not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof; and

h) evaluating the activity and the retention of at least one other characteristic or property of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-  
15 binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

In another embodiment the invention provides a method to improve the affinity of an antibody or antigen-binding portion thereof, comprising:

20 a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;

b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A,  
25 H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;

30 d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other characteristic or property until an antibody, or antigen-binding portion thereof, with an improved activity, relative to the parent antibody, or antigen-binding  
5 portion thereof, is obtained.

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

a) providing a parent antibody or antigen-binding portion thereof;  
b) selecting an amino acid residue within a complementarity determining region  
10 (CDR) for mutation at a position other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions  
15 thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies or antigen-binding portions thereof,  
20 relative to the parent antibody or antigen-portion thereof, for changes in at least one other property or characteristic;

f) repeating steps b) through e) for at least one other CDR position which is neither the position selected under b) nor a position at H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32,  
25 L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

g) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity but not affecting at least one other property or characteristic, to form combination antibodies, or antigen-binding portions thereof with at least one retained property or  
30 characteristic; and

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h) evaluating the activity and the retention of at least one property of characteristic of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one retained property or characteristic, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce an antibody with a close to germline immunoglobulin sequence

In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, without affecting other characteristics, comprising:

a) providing a parent antibody or antigen-binding portion thereof;  
b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;

c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof;

d) evaluating the activity of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof thereby identifying an activity enhancing amino acid residue;

e) evaluating the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, for changes in at least one other property or characteristic until an antibody, or antigen-binding portion thereof, with an improved activity and retained other characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

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In another embodiment, the invention provides a method for improving the activity of an antibody, or antigen-binding portion thereof, comprising:

- a) providing a parent antibody or antigen-binding portion thereof that was obtained by selection in a phage-display system but whose activity cannot be further improved by mutagenesis in said phage-display system;
- b) selecting an amino acid residue within a complementarity determining region (CDR) for mutation other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- c) individually mutating said selected position to at least two other amino acid residues to thereby create a panel of mutated antibodies, or antigen-binding portions thereof and expression in a non-phage display system;
- d) evaluating the activity and retention of at least one other characteristic or property of the panel of mutated antibodies, or antigen-binding portions thereof, relative to the parent antibody or antigen-binding portion thereof, thereby identifying an activity enhancing amino acid residue;
- e) repeating steps b) through d) for at least one other CDR position which is neither the position selected under b nor other than H30, H31, H31B, H32, H33, H35, H50, H52, H52A, H53, H54, H56, H58, H95, H96, H97, H98, H101, L30, L31, L32, L34, L50, L52, L53, L55, L91, L92, L93, L94 and L96;
- f) combining, in the parent antibody, or antigen-binding portion thereof, at least two individual activity enhancing amino acid residues shown to have improved activity and not to affect at least one other characteristic or property, to form combination antibodies, or antigen-binding portions thereof; and
- g) evaluating the activity and retention of at least one other characteristic or property of the combination antibodies, or antigen-binding portions thereof with two activity enhancing amino acid residues, relative to the parent antibody or antigen-binding portion thereof until an antibody, or antigen-binding portion thereof, with an improved activity and at least one other retained characteristic or property, relative to the parent antibody, or antigen-binding portion thereof, is obtained.

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Preferably, the other characteristic or property is selected from 1) preservation of non-crossreactivity with other proteins or human tissues, 2) preservation of epitope recognition, i.e. recognizing p40 epitope preferably in the context of the p70 p40/p35 heterodimer preventing binding interference from free, soluble p40 and/or 3) to produce  
5 an antibody with a close to germline immunoglobulin sequence

### Brief Description of the Drawings

Figures 1A-1B show the heavy chain variable region amino acid sequence alignments of a series of human antibodies that bind human IL-12 compared to germline  
10 sequences Cos-3/JH3 and Dpl18 Lv1042. Kabat numbering is used to identify amino acid positions. For the Joe 9 wild type, the full sequence is shown. For the other antibodies, only those amino acids positions that differ from Joe 9 wild type are shown.

Figures 1C-1D show the light chain variable region amino acid sequence alignments of a series of human antibodies that bind human IL-12. Kabat numbering is  
15 used to identify amino acid positions. For the Joe 9 wild type, the full sequence is shown. For the other antibodies, only those amino acids positions that differ from Joe 9 wild type are shown.

Figures 2A-2E show the CDR positions in the heavy chain of the Y61 antibody that were mutated by site-directed mutagenesis and the respective amino acid  
20 substitutions at each position. The graphs at the right of the figures show the off-rates for the substituted antibodies (black bars) as compared to unmutated Y61 (open bar).

Figures 2F-2H show the CDR positions in the light chain of the Y61 antibody that were mutated by site-directed mutagenesis and the respective amino acid  
25 substitutions at each position. The graphs at the right of the figures show the off-rates for the substituted antibodies (black bars) as compared to unmutated Y61 (open bar).

Figure 3 demonstrates the *in vivo* efficacy of the human anti-IL-12 antibody J695, on plasma neopterin levels in cynomolgus monkeys.

Figure 4 shows a graph of mean arthritic score versus days after immunization of mice with collagen, demonstrating that treatment with C17.15 significantly decreases  
30 arthritis-related symptoms as compared to treatment with rat IgG.

### Detailed Description of the Invention

In order that the present invention may be more readily understood, certain terms are first defined.

The term "activity enhancing amino acid residue" includes an amino acid residue which improves the activity of the antibody. It should be understood that the activity enhancing amino acid residue may replace an amino acid residue at a contact, hypermutation or preferred selective mutagenesis position and, further, more than one activity enhancing amino acid residue can be present within one or more CDRs. An activity enhancing amino acid residue include, an amino acid residue that improves the binding specificity/affinity of an antibody, for example anti-human IL-12 antibody binding to human IL-12. The activity enhancing amino acid residue is also intended to include an amino acid residue that improves the neutralization potency of an antibody, for example, the human IL-12 antibody which inhibits human IL-12.

The term "antibody" includes an immunoglobulin molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4.

The term "antigen-binding portion" of an antibody (or "antibody portion") includes fragments of an antibody that retain the ability to specifically bind to an antigen (e.g., hIL-12). It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a F(ab')<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a



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disulfide bridge at the hinge region: (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546 ), which consists of a VH domain; and (vi) an isolated complementarity determining region (CDR).

- 5 Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv): see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883) . Such single chain antibodies are also intended to be encompassed within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the
- 10 same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecules, formed by covalent or non-covalent association of the
- 15 antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and F(ab')<sub>2</sub> fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein. Preferred antigen binding portions
- 20 are complete domains or pairs of complete domains.
- 25
- 30

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The term "backmutation" refers to a process in which some or all of the somatically mutated amino acids of a human antibody are replaced with the corresponding germline residues from a homologous germline antibody sequence. The heavy and light chain sequences of the human antibody of the invention are aligned separately with the germline sequences in the VBASE database to identify the sequences with the highest homology. Differences in the human antibody of the invention are returned to the germline sequence by mutating defined nucleotide positions encoding such different amino acid. The role of each amino acid thus identified as candidate for backmutation should be investigated for a direct or indirect role in antigen binding and any amino acid found after mutation to affect any desirable characteristic of the human antibody should not be included in the final human antibody; as an example, activity enhancing amino acids identified by the selective mutagenesis approach will not be subject to backmutation. To minimize the number of amino acids subject to backmutation those amino acid positions found to be different from the closest germline sequence but identical to the corresponding amino acid in a second germline sequence can remain, provided that the second germline sequence is identical and colinear to the sequence of the human antibody of the invention for at least 10, preferably 12 amino acids, on both sides of the amino acid in question. Backmutation may occur at any stage of antibody optimization; preferably, backmutation occurs directly before or after the selective mutagenesis approach. More preferably, backmutation occurs directly before the selective mutagenesis approach.

The phrase "human interleukin 12" (abbreviated herein as hIL-12, or IL-12), as used herein, includes a human cytokine that is secreted primarily by macrophages and dendritic cells. The term includes a heterodimeric protein comprising a 35 kD subunit (p35) and a 40 kD subunit (p40) which are both linked together with a disulfide bridge. The heterodimeric protein is referred to as a "p70 subunit". The structure of human IL-12 is described further in, for example, Kobayashi, *et al.* (1989) *J. Exp Med.* 170:827-845; Seder, *et al.* (1993) *Proc. Natl. Acad. Sci.* 90:10188-10192; Ling, *et al.* (1995) *J. Exp Med.* 154:116-127; Podlaski, *et al.* (1992) *Arch. Biochem. Biophys.* 294:230-237. The term human IL-12 is intended to include recombinant human IL-12 (rh IL-12), which can be prepared by standard recombinant expression methods.

The terms "Kabat numbering", "Kabat definitions and "Kabat labeling" are used interchangeably herein. These terms, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (*i.e.* hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad. Sci.* 190:382-391 and , Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

The Kabat numbering is used herein to indicate the positions of amino acid modifications made in antibodies of the invention. For example, the Y61 anti-IL-12 antibody can be mutated from serine (S) to glutamic acid (E) at position 31 of the heavy chain CDR1 (H31S → E), or glycine (G) can be mutated to tyrosine (Y) at position 94 of the light chain CDR3 (L94G → Y).

The term "human antibody" includes antibodies having variable and constant regions corresponding to human germline immunoglobulin sequences as described by Kabat *et al.* (See Kabat, *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs and in particular CDR3. The mutations preferably are introduced using the "selective mutagenesis approach" described herein. The human antibody can have at least one position replaced with an amino acid residue, *e.g.*, an activity enhancing amino acid residue which is not encoded by the human germline immunoglobulin sequence. The human antibody can have up to twenty positions replaced with amino acid residues which are not part of the human germline immunoglobulin sequence. In other embodiments, up to ten, up to five, up to three or up to two positions are replaced. In a preferred embodiment, these replacements are within

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the CDR regions as described in detail below. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

5           The phrase "recombinant human antibody" includes human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described further in Section II, below), antibodies isolated from a recombinant, combinatorial human antibody library (described further in Section III, below), antibodies isolated  
10 from an animal (*e.g.*, a mouse) that is transgenic for human immunoglobulin genes (see *e.g.*, Taylor, L.D., *et al.* (1992) *Nucl. Acids Res.* 20:6287-6295) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline  
15 immunoglobulin sequences (See Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*. U.S. Department of Health and Human Services, NIH Publication No. 91-3242). In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid  
20 sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire *in vivo*. In certain embodiments, however, such recombinant antibodies are the result of selective mutagenesis approach or backmutation or both.

25           An "isolated antibody" includes an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds hIL-12 is substantially free of antibodies that specifically bind antigens other than hIL-12). An isolated antibody that specifically binds hIL-12 may bind IL-12 molecules from other species (discussed in further detail below). Moreover,  
30 an isolated antibody may be substantially free of other cellular material and/or chemicals.

A "neutralizing antibody" (or an "antibody that neutralized hIL-12 activity") includes an antibody whose binding to hIL-12 results in inhibition of the biological activity of hIL-12. This inhibition of the biological activity of hIL-12 can be assessed by measuring one or more indicators of hIL-12 biological activity, such as inhibition of human phytohemagglutinin blast proliferation in a phytohemagglutinin blast proliferation assay (PHA), or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3-Interferon-gamma Induction Assay). These indicators of hIL-12 biological activity can be assessed by one or more of several standard *in vitro* or *in vivo* assays known in the art (see Example 3).

10       The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen, for example, an anti-hIL-12 antibody that binds to an IL-12 antigen and/or the neutralizing potency of an antibody, for example, an anti-hIL-12 antibody whose binding to hIL-12 inhibits the biological activity of hIL-12, e.g. inhibition of PHA blast proliferation or inhibition of receptor binding in a human IL-12 receptor binding assay (see Example 3).

15       The phrase "surface plasmon resonance" includes an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Example 5 and Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnsson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

      The term " $K_{off}$ ", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex.

25       The term " $K_d$ ", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction.

      The phrase "nucleic acid molecule" includes DNA molecules and RNA molecules. A nucleic acid molecule may be single-stranded or double-stranded, but preferably is double-stranded DNA.

30       The phrase "isolated nucleic acid molecule", as used herein in reference to nucleic acids encoding antibodies or antibody portions (e.g., VH, VL, CDR3) that bind hIL-12 including "isolated antibodies"), includes a nucleic acid molecule in which the

nucleotide sequences encoding the antibody or antibody portion are free of other nucleotide sequences encoding antibodies or antibody portions that bind antigens other than hIL-12, which other sequences may naturally flank the nucleic acid in human genomic DNA. Thus, for example, an isolated nucleic acid of the invention encoding a  
5 VII region of an anti-IL-12 antibody contains no other sequences encoding other VH regions that bind antigens other than IL-12. The phrase "isolated nucleic acid molecule" is also intended to include sequences encoding bivalent, bispecific antibodies, such as diabodies in which VH and VL regions contain no other sequences other than the sequences of the diabody.

10 The term "vector" includes a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of  
15 autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes  
20 to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However,  
25 the invention is intended to include such other forms of expression vectors, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The phrase "recombinant host cell" (or simply "host cell") includes a cell into which a recombinant expression vector has been introduced. It should be understood  
30 that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not,

in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein.

The term "modifying", as used herein, is intended to refer to changing one or more amino acids in the antibodies or antigen-binding portions thereof. The change can be produced by adding, substituting or deleting an amino acid at one or more positions. The change can be produced using known techniques, such as PCR mutagenesis.

The phrase "contact position" includes an amino acid position of in the CDR1, CDR2 or CDR3 of the heavy chain variable region or the light chain variable region of an antibody which is occupied by an amino acid that contacts antigen in one of the twenty-six known antibody-antigen structures. If a CDR amino acid in any of the 26 known solved structures of antibody-antigen complexes contacts the antigen, then that amino acid can be considered to occupy a contact position. Contact positions have a higher probability of being occupied by an amino acid which contact antigen than non-contact positions. Preferably a contact position is a CDR position which contains an amino acid that contacts antigen in greater than 3 of the 26 structures (>11.5 %). Most preferably a contact position is a CDR position which contains an amino acid that contacts antigen in greater than 8 of the 25 structures (>32%).

The term "hypermutation position" includes an amino acid residue that occupies position in the CDR1, CDR2 or CDR3 region of the heavy chain variable region or the light chain variable region of an antibody that is considered to have a high frequency or probability for somatic hypermutation during *in vivo* affinity maturation of the antibody. "High frequency or probability for somatic hypermutation" includes frequencies or probabilities of a 5 to about 40% chance that the residue will undergo somatic hypermutation during *in vivo* affinity maturation of the antibody. It should be understood that all ranges within this stated range are also intended to be part of this invention, e.g., 5 to about 30%, e.g., 5 to about 15%, e.g., 15 to about 30%.

The term "preferred selective mutagenesis position" includes an amino acid residue that occupies a position in the CDR1, CDR2 or CDR3 region of the heavy chain variable region or the light chain variable region which can be considered to be both a contact and a hypermutation position.

The phrase "selective mutagenesis approach" includes a method of improving the activity of an antibody by selecting and individually mutating CDR amino acids at at least one preferred selective mutagenesis position, hypermutation, and/or contact position. A "selectively mutated" human antibody is an antibody which contains, a mutation at a position selected using a selective mutagenesis approach. In another embodiment, the selective mutagenesis approach is intended to provide a method of preferentially mutating selected individual amino acid residues in the CDR1, CDR2 or CDR3 of the heavy chain variable region (hereinafter H1, H2, and H3, respectively), or the CDR1, CDR2 or CDR3 of the light chain variable region (hereinafter referred to as L1, L2, and L3, respectively) of an antibody. Amino acid residues may be selected from preferred selective mutagenesis positions, contact positions, or hypermutation positions. Individual amino acids are selected based on their position in the light or heavy chain variable region. It should be understood that a hypermutation position can also be a contact position. In an embodiment, the selective mutagenesis approach is a "targeted approach". The language "targeted approach" is intended to include a method of preferentially mutating selected individual amino acid residues in the CDR1, CDR2 or CDR3 of the heavy chain variable region or the CDR1, CDR2 or CDR3 of the light chain variable region of an antibody in a targeted manner, *e.g.*, a "Group-wise targeted approach" or "CDR-wise targeted approach". In the "Group-wise targeted approach", individual amino acid residues in particular groups are targeted for selective mutations including groups I (including L3 and H3), II (including H2 and L1) and III (including L2 and H1), the groups being listed in order of preference for targeting. In the "CDR-wise targeted approach", individual amino acid residues in particular CDRs are targeted for selective mutations with the order of preference for targeting as follows: H3, L3, H2, L1, H1 and L2. The selected amino acid residue is mutated, *e.g.*, to at least two other amino acid residues, and the effect of the mutation on the activity of the antibody is determined. Activity is measured as a change in the binding specificity/affinity of the antibody, and/or neutralization potency of the antibody. It should be understood that the selective mutagenesis approach can be used for the optimization of any antibody derived from any source including phage display, transgenic animals with human IgG germline genes, human antibodies isolated from human B-cells. Preferably, the selective mutagenesis approach is used on antibodies which can not be optimized further using



phage display technology. It should be understood that antibodies from any source including phage display, transgenic animals with human IgG germline genes, human antibodies isolated from human B-cells can be subject to backmutation prior to or after the selective mutagenesis approach.

- 5           The term "activity enhancing amino acid residue" includes an amino acid residue which improves the activity of the antibody. It should be understood that the activity enhancing amino acid residue may replace an amino acid residue at a preferred selective mutagenesis position, contact position, or a hypermutation position and, further, more than one activity enhancing amino acid residue can be present within one or more CDRs.
- 10   An activity enhancing amino acid residue include, an amino acid residue that improves the binding specificity/affinity of an antibody, for example anti-human IL-12 antibody binding to human IL-12. The activity enhancing amino acid residue is also intended to include an amino acid residue that improves the neutralization potency of an antibody, for example, the human IL-12 antibody which inhibits human IL-12.

- 15           Various aspects of the invention are described in further detail in the following subsections.

I.    Human Antibodies that Bind Human IL-12

- This invention provides isolated human antibodies, or antigen-binding portions thereof, that bind to human IL-12. Preferably, the human antibodies of the invention are
- 20   recombinant, neutralizing human anti-hIL-12 antibodies. Antibodies of the invention that bind to human IL-12 can be selected, for example, by screening one or more human  $V_L$  and  $V_H$  cDNA libraries with hIL-12, such as by phage display techniques as described in Example 1. Screening of human  $V_L$  and  $V_H$  cDNA libraries initially identified a series of anti-IL-12 antibodies of which one antibody, referred to herein as
- 25   "Joe 9" (or "Joe 9 wild type"), was selected for further development. Joe 9 is a relatively low affinity human IL-12 antibody (*e.g.*, a  $K_{off}$  of about  $0.1 \text{ sec}^{-1}$ ), yet is useful for specifically binding and detecting hIL-12. The affinity of the Joe 9 antibody was improved by conducting mutagenesis of the heavy and light chain CDRs, producing a panel of light and heavy chain variable regions that were "mixed and matched" and
- 30   further mutated, leading to numerous additional anti-hIL-12 antibodies with increased affinity for hIL-12 (see Example 1, Table 2 (see Appendix A) and the sequence alignments of Figures 1A-D).

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Of these antibodies, the human anti-hIL-12 antibody referred to herein as Y61 demonstrated a significant improvement in binding affinity (e.g., a  $K_{off}$  of about  $2 \times 10^{-4}$  sec<sup>-1</sup>). The Y61 anti-hIL-12 antibody was selected for further affinity maturation by individually mutating specific amino acids residues within the heavy and light chain CDRs. Amino acids residues of Y61 were selected for site-specific mutation (selective mutagenesis approach) based on the amino acid residue occupying a preferred selective mutagenesis position, contact and/or a hypermutation position. A summary of the substitutions at selected positions in the heavy and light chain CDRs is shown in Figures 2A-2H. A preferred recombinant neutralizing antibody of the invention, referred to herein as J695, resulted from a Gly to Tyr substitution at position 50 of the light chain CDR2 of Y61, and a Gly to Tyr substitution at position 94 of the light chain CDR3 of Y61.

Amino acid sequence alignments of the heavy and light chain variable regions of a panel of anti-IL-12 antibodies of the invention, on the lineage from Joe 9 wild type to J695, are shown in Figures 1A-1D. These sequence alignments allowed for the identification of consensus sequences for preferred heavy and light chain variable regions of antibodies of the invention that bind hIL-12, as well as consensus sequences for the CDR3, CDR2, and CDR1, on the lineage from Joe 9 to J695. Moreover, the Y61 mutagenesis analysis summarized in Figures 2A-2H allowed for the identification of consensus sequences for heavy and light chain variable regions that bind hIL-12, as well as consensus sequences for the CDR3, CDR2, and CDR1 that bind hIL-12 on the lineage from Y61 to J695 that encompasses sequences with modifications from Y61 yet that retain good hIL-12 binding characteristics. Preferred CDR, VH and VL sequences of the invention (including consensus sequences) as identified by sequence identifiers in the attached Sequence Listing, are summarized below.

SEQ ID NO:	ANTIBODY CHAIN	REGION	SEQUENCE
1	Consensus Joe 9 to J695	CDR H3	(H/S) -G-S- (H/Y) -D- (N/T/Y)
2	Consensus	CDR L3	Q- (S/T) -Y- (D/E) - (S/R/K) - (S/G/Y) -

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	Joe 9 to J695		(L/F/T/S) - (R/S/T/W/H) - (G/P) - (S/T/A/L) - (R/S/M/T/L) - (V/I/T/M/L)
3	Consensus Joe 9 to J695	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
4	Consensus Joe 9 to J695	CDR L2	(G/Y) - N - (D/S) - (Q/N) - R - P - S
5	Consensus Joe 9 to J695	CDR H1	F-T-F-S - (S/E) - Y-G-M-H
6	Consensus Joe 9 to J695	CDR L1	(S/T) - G - (G/S) - (R/S) - S-N-I - (G/V) - (S/A) - (N/G/Y) - (T/D) - V - (K/H)
7	Consensus Joe 9 to J695	VH	(full VH sequence; see sequence listing)
8	Consensus Joe 9 to J695	VL	(full VL sequence; see sequence listing)
9	Consensus Y61 to J695	CDR H3	H - (G/V/C/H) - (S/T) - (H/T/V/R/I) - (D/S) - (N/K/A/T/S/F/W/H)
10	Consensus Y61 to J695	CDR L3	Q-S-Y - (D/S) - (Xaa) - (G/D/Q/L/F/R/H/N/Y) - T-H-P-A-L-L
11	Consensus Y61 to J695	CDR H2	(F/T/Y) - I - (R/A) - Y - (D/S/E/A) - (G/R) - S - (Xaa) - K - (Y/E) - Y-A-D-S-V-K-G
12	Consensus Y61 to J695	CDR L2	(G/Y/S/T/N/Q) - N-D-Q-R-P-S
13	Consensus Y61 to J695	CDR H1	F-T-F - (Xaa) - (Xaa) - (Y/H) - (G/M/A/N/S) - M-H
14	Consensus Y61 to J695	CDR L1	S-G-G-R-S-N-I-G - (S/C/R/N/D/T) - (N/M/I) - (T/Y/D/H/K/P) - V-K
15	Consensus Y61 to J695	VH	(full VH sequence; see sequence listing)
16	Consensus Y61 to J695	VL	(full VL sequence; see sequence listing)
17	Y61	CDR H3	H-G-S-H-D-N
18	Y61	CDR L3	Q-S-Y-D-R-G-T-H-P-A-L-L
19	Y61	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
20	Y61	CDR L2	G-N-D-Q-R-P-S
21	Y61	CDR H1	F-T-F-S-S-Y-G-M-H
22	Y61	CDR L1	S-G-G-R-S-N-I-G-S-N-T-V-K

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23	Y61	VH	(full VH sequence; see sequence listing)
24	Y61	VL	(full VL sequence; see sequence listing)
25	J695	CDR H3	H-G-S-H-D-N
26	J695	CDR L3	Q-S-Y-D-R-Y-T-H-P-A-L-L
27	J695	CDR H2	F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G
28	J695	CDR L2	Y-N-D-Q-R-P-S
29	J695	CDR H1	F-T-F-S-S-Y-G-M-H
30	J695	CDR L1	S-G-S-R-S-N-I-G-S-N-T-V-K
31	J695	VH	(full VH sequence; see sequence listing)
32	J695	VL	(full VL sequence; see sequence listing)

Antibodies produced from affinity maturation of Joe 9 wild type were functionally characterized by surface plasmon resonance analysis to determine the  $K_d$  and  $K_{off}$  rate. A series of antibodies were produced having a  $K_{off}$  rate within the range of about  $0.1 \text{ s}^{-1}$  to about  $1 \times 10^{-5} \text{ s}^{-1}$ , and more preferably a  $K_{off}$  of about  $1 \times 10^{-4} \text{ s}^{-1}$  to  $1 \times 10^{-5} \text{ s}^{-1}$  or less. Antibodies were also characterized *in vitro* for their ability to inhibit phytohemagglutinin (PHA) blast proliferation, as described in Example 3. A series of antibodies were produced having an  $IC_{50}$  value in the range of about  $1 \times 10^{-6} \text{ M}$  to about  $1 \times 10^{-11} \text{ M}$ , more preferably about  $1 \times 10^{-10} \text{ M}$  to  $1 \times 10^{-11} \text{ M}$  or less.

Accordingly, in one aspect, the invention provides an isolated human antibody, or antigen-binding portion thereof, that binds to human IL-12 and dissociates from human IL-12 with a  $K_{off}$  rate constant of  $0.1 \text{ s}^{-1}$  or less, as determined by surface plasmon resonance, or which inhibits phytohemagglutinin blast proliferation in an *in vitro* phytohemagglutinin blast proliferation assay (PHA assay) with an  $IC_{50}$  of  $1 \times 10^{-6} \text{ M}$  or less. In preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-2} \text{ s}^{-1}$  or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-7} \text{ M}$  or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-3} \text{ s}^{-1}$  or less, or inhibits phytohemagglutinin

blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-8}$  M or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-4}$  s<sup>-1</sup> or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less. In more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-5}$  s<sup>-1</sup> or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-10}$  M or less. In even more preferred embodiments, the isolated human IL-12 antibody, or an antigen-binding portion thereof, dissociates from human IL-12 with a  $K_{off}$  rate constant of  $1 \times 10^{-5}$  s<sup>-1</sup> or less, or inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-11}$  M or less.

The dissociation rate constant ( $K_{off}$ ) of an IL-12 antibody can be determined by surface plasmon resonance (see Example 5). Generally, surface plasmon resonance analysis measures real-time binding interactions between ligand (recombinant human IL-12 immobilized on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). Surface plasmon analysis can also be performed by immobilizing the analyte (antibodies on a biosensor matrix) and presenting the ligand (recombinant IL-12 in solution). Neutralization activity of IL-12 antibodies, or antigen binding portions thereof, can be assessed using one or more of several suitable *in vitro* assays (see Example 3).

It is well known in the art that antibody heavy and light chain CDRs play an important role in the binding specificity/affinity of an antibody for an antigen. Accordingly, the invention encompasses human antibodies having light and heavy chain CDRs of Joe 9, as well as other antibodies having CDRs that have been modified to improve the binding specificity/affinity of the antibody. As demonstrated in Example 1, a series of modifications to the light and heavy chain CDRs results in affinity maturation of human anti-hIL-12 antibodies. The heavy and light chain variable region amino acid sequence alignments of a series of human antibodies ranging from Joe 9 wild type to J695 that bind human IL-12 is shown in Figures 1A-1D. Consensus sequence motifs for the CDRs of antibodies can be determined from the sequence alignment (as

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summarized in the table above). For example, a consensus motif for the VH CDR3 of the lineage from Joe 9 to J695 comprises the amino acid sequence: (H/S)-G-S-(H/Y)-D-(N/T/Y) (SEQ ID NO: 1), which encompasses amino acids from position 95 to 102 of the consensus HCVR shown in SEQ ID NO: 7. A consensus motif for the VL CDR3  
 5 comprises the amino acid sequence: Q-(S/T)-Y-(D/E)-(S/R/K)-(S/G/Y)-(L/F/T/S)-(R/S/T/W/H)-(G/P)-(S/T/A/L)-(R/S/M/T/L-V/I/T/M/L) (SEQ ID NO: 2), which encompasses amino acids from position 89 to 97 of the consensus LCVR shown in SEQ ID NO: 8.

Accordingly, in another aspect, the invention provides an isolated human  
 10 antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC<sub>50</sub> of  $1 \times 10^{-6}$  M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 1; and
- 15 c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 2.

In a preferred embodiment, the antibody further comprises a VH CDR2 comprising the amino acid sequence: F-I-R-Y-D-G-S-N-K-Y-Y-A-D-S-V-K-G (SEQ ID NO: 3) (which encompasses amino acids from position 50 to 65 of the consensus  
 20 HCVR comprising the amino acid sequence SEQ ID NO: 7) and further comprises a VL CDR2 comprising the amino acid sequence: (G/Y)-N-(D/S)-(Q/N)-R-P-S (SEQ ID NO: 4) (which encompasses amino acids from position 50 to 56 of the consensus LCVR comprising the amino acid sequence SEQ ID NO: 8).

In another preferred embodiment, the antibody further comprises a VH CDR1  
 25 comprising the amino acid sequence: F-T-F-S-(S/E)-Y-G-M-H (SEQ ID NO: 5) (which encompasses amino acids from position 27 to 35 of the consensus HCVR comprising the amino acid sequence SEQ ID NO: 7) and further comprises a VL CDR1 comprising the amino acid sequence: (S/T)-G-(G/S)-(R/S)-S-N-I-(G/V)-(S/A)-(N/G/Y)-(T/D)-V-(K/H) (SEQ ID NO: 6) (which encompasses amino acids from position 24 to 34 of the  
 30 consensus LCVR comprising the amino acid sequence SEQ ID NO: 8).

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In yet another preferred embodiment, the antibody of the invention comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 7 and a LCVR comprising the amino acid sequence of SEQ ID NO: 8.

Additional consensus motifs can be determined based on the mutational analysis performed on Y61 that led to the J695 antibody (summarized in Figures 2A-2H). As demonstrated by the graphs shown in Figures 2A-2H, certain residues of the heavy and light chain CDRs of Y61 were amenable to substitution without significantly impairing the hIL-12 binding properties of the antibody. For example, individual substitutions at position 30 in CDR H1 with twelve different amino acid residues did not significantly reduce the  $K_{off}$  rate of the antibody, indicating that this position is amenable to substitution with a variety of different amino acid residues. Thus, based on the mutational analysis (i.e., positions within Y61 that were amenable to substitution by other amino acid residues) consensus motifs were determined. The consensus motifs for the heavy and light chain CDR3s are shown in SEQ ID NOs: 9 and 10, respectively, consensus motifs for the heavy and light chain CDR2s are shown in SEQ ID NOs: 11 and 12, respectively, and consensus motifs for the heavy and light chain CDR1s are shown in SEQ ID NOs: 13 and 14, respectively. Consensus motifs for the VH and VL regions are shown in SEQ ID NOs: 15 and 16, respectively.

Accordingly, in one aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which has the following characteristics:

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 9; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 10.

In a preferred embodiment, the antibody further comprises a VH CDR2 comprising the amino acid sequence of SEQ ID NO: 11 and further comprises a VL CDR2 comprising the amino acid sequence of SEQ ID NO: 12.

In another preferred embodiment, the antibody further comprises a VH CDR1 comprising the amino acid sequence of SEQ ID NO: 13 and further comprises a VL CDR1 comprising the amino acid sequence of SEQ ID NO: 14.

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In yet another preferred embodiment, the antibody of the invention comprises a HCVR comprising the amino acid sequence of SEQ ID NO: 15 and a LCVR comprising the amino acid sequence of SEQ ID NO: 16.

A preferred antibody of the invention, the human anti-hIL-12 antibody Y61, was produced by affinity maturation of Joe 9 wild type by PCR mutagenesis of the CDR3 (as described in Example 1). Y61 had an improved specificity/binding affinity determined by surface plasmon resonance and by *in vitro* neutralization assays. The heavy and light chain CDR3s of Y61 are shown in SEQ ID NOs: 17 and 18, respectively, the heavy and light chain CDR2s of Y61 are shown in SEQ ID NOs: 19 and 20, respectively, and the heavy and light chain CDR1s of Y61 are shown in SEQ ID NOs: 21 and 22, respectively. The VH of Y61 has the amino acid sequence of SEQ ID NO: 23 and the VL of Y61 has the amino acid sequence of SEQ ID NO: 24 (these sequences are also shown in Figures 1A-1D, aligned with Joe9).

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an  $IC_{50}$  of  $1 \times 10^{-9}$  M or less;
- b) has a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 17; and
- c) has a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 18.

In a preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, has a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 19 and a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 20.

In another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof has a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 21 and a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 22.



In yet another preferred embodiment, the isolated human antibody, or an antigen-binding portion thereof, comprising a the heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 23, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 24.

5 In certain embodiments, the full length antibody comprises a heavy chain constant region, such as IgG1, IgG2, IgG3, IgG4, IgM, IgA and IgE constant regions, and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). Preferably, the antibody  
10 heavy chain constant region is an IgG1 heavy chain constant region. Alternatively, the antibody portion can be an Fab fragment, an F(ab')<sub>2</sub> fragment or a single chain Fv fragment.

Modifications of individual residues of Y61 led to the production of a panel of antibodies shown in Figures 2A-2H. The specificity/binding affinity of each antibody  
15 was determined by surface plasmon resonance and/or by *in vitro* neutralization assays.

Accordingly, in another aspect, the invention features an isolated human antibody, or an antigen-binding portion thereof, which

- a) inhibits phytohemagglutinin blast proliferation in an *in vitro* PHA assay with an IC<sub>50</sub> of  $1 \times 10^{-9}$  M or less;
- 20 b) has a heavy chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 404-SEQ ID NO: 469; and
- c) has a light chain CDR3 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 534-SEQ ID NO: 579.

In preferred embodiment, the isolated human antibody, or an antigen-binding  
25 portion thereof, has a heavy chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO:335-SEQ ID NO: 403; and a light chain CDR2 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 506-SEQ ID NO: 533.

In another preferred embodiment, the isolated human antibody, or an antigen-  
30 binding portion thereof, has a heavy chain CDR1 comprising the amino acid sequence selected from the group consisting of SEQ ID NO: 288-SEQ ID NO: 334; and a light

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chain CDR1 comprising the amino acid sequence selected from the group consisting of  
SEQ ID NO: 470-SEQ ID NO: 505.

In yet another preferred embodiment, the isolated human antibody, or an antigen-

(1989), Ausubel, F.M. *et al.* (eds.) *Current Protocols in Molecular Biology*, Greene Publishing Associates, (1989) and in U.S. Patent No. 4,816,397 by Boss *et al.*

To obtain a DNA fragment encoding the heavy chain variable region of Joe 9 wt or a Joe 9 wt-related antibody, antibodies specific for human IL-12 were screened from human libraries and mutated, as described in section II. Once DNA fragments encoding Joe 9 wt or Joe 9 wt-related VH and VL segments are obtained, mutagenesis of these sequences is carried out by standard methods, such as PCR site directed mutagenesis (PCR-mediated mutagenesis in which the mutated nucleotides are incorporated into the PCR primers such that the PCR product contains the mutations) or other site-directed mutagenesis methods. Human IL-12 antibodies that displayed a level of activity and binding specificity/affinity that was desirable, for example J695, were further manipulated by standard recombinant DNA techniques, for example to convert the variable region genes to full-length antibody chain genes, to Fab fragment genes or to a scFv gene. In these manipulations, a VL- or VH-encoding DNA fragment is operatively linked to another DNA fragment encoding another protein, such as an antibody constant region or a flexible linker. The term "operatively linked", as used in this context, is intended to mean that the two DNA fragments are joined such that the amino acid sequences encoded by the two DNA fragments remain in-frame.

The isolated DNA encoding the VH region can be converted to a full-length heavy chain gene by operatively linking the VH-encoding DNA to another DNA molecule encoding heavy chain constant regions (CH1, CH2 and CH3). The sequences of human heavy chain constant region genes are known in the art (see *e.g.*, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The heavy chain constant region can be an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region and any allotypic variant therein as described in Kabat (, Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242), but most preferably is an IgG1 or IgG4 constant region. For a Fab fragment heavy chain gene, the VH-encoding DNA can be operatively linked to another DNA molecule encoding only the heavy chain CH1 constant region.

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The isolated DNA encoding the VL region can be converted to a full-length light chain gene (as well as a Fab light chain gene) by operatively linking the VL-encoding DNA to another DNA molecule encoding the light chain constant region, CL. The sequences of human light chain constant region genes are known in the art (see *e.g.*,

5 Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242) and DNA fragments encompassing these regions can be obtained by standard PCR amplification. The light chain constant region can be a kappa or lambda constant region, but most preferably is a lambda constant region.

10 To create a scFv gene, the VH- and VL-encoding DNA fragments are operatively linked to another fragment encoding a flexible linker, *e.g.*, encoding the amino acid sequence (Gly<sub>4</sub>-Ser)<sub>3</sub>, such that the VH and VL sequences can be expressed as a contiguous single-chain protein, with the VL and VH regions joined by the flexible linker (see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; Huston *et al.* (1988) *Proc. Natl.*  
15 *Acad. Sci. USA* 85:5879-5883; McCafferty *et al.*, *Nature* (1990) 348:552-554).

To express the antibodies, or antibody portions of the invention, DNAs encoding partial or full-length light and heavy chains, obtained as described above, are inserted into expression vectors such that the genes are operatively linked to transcriptional and translational control sequences. In this context, the term "operatively linked" is intended  
20 to mean that an antibody gene is ligated into a vector such that transcriptional and translational control sequences within the vector serve their intended function of regulating the transcription and translation of the antibody gene. The expression vector and expression control sequences are chosen to be compatible with the expression host cell used. The antibody light chain gene and the antibody heavy chain gene can be  
25 inserted into separate vector or, more typically, both genes are inserted into the same expression vector. The antibody genes are inserted into the expression vector by standard methods (*e.g.*, ligation of complementary restriction sites on the antibody gene fragment and vector, or blunt end ligation if no restriction sites are present). Prior to insertion of the J695 or J695-related light or heavy chain sequences, the expression  
30 vector may already carry antibody constant region sequences. For example, one approach to converting the J695 or J695-related VH and VL sequences to full-length antibody genes is to insert them into expression vectors already encoding heavy chain

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constant and light chain constant regions, respectively, such that the VH segment is operatively linked to the CH segment(s) within the vector and the VL segment is operatively linked to the CL segment within the vector. Additionally or alternatively, the recombinant expression vector can encode a signal peptide that facilitates secretion of the antibody chain from a host cell. The antibody chain gene can be cloned into the vector such that the signal peptide is linked in-frame to the amino terminus of the antibody chain gene. The signal peptide can be an immunoglobulin signal peptide or a heterologous signal peptide (*i.e.*, a signal peptide from a non-immunoglobulin protein).

In addition to the antibody chain genes, the recombinant expression vectors of the invention carry regulatory sequences that control the expression of the antibody chain genes in a host cell. The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals) that control the transcription or translation of the antibody chain genes. Such regulatory sequences are described, for example, in Goeddel; *Gene Expression Technology: Methods in Enzymology* 185, Academic Press, San Diego, CA (1990). It will be appreciated by those skilled in the art that the design of the expression vector, including the selection of regulatory sequences may depend on such factors as the choice of the host cell to be transformed, the level of expression of protein desired, *etc.* Preferred regulatory sequences for mammalian host cell expression include viral elements that direct high levels of protein expression in mammalian cells, such as promoters and/or enhancers derived from cytomegalovirus (CMV) (such as the CMV promoter/enhancer), Simian Virus 40 (SV40) (such as the SV40 promoter/enhancer), adenovirus, (*e.g.*, the adenovirus major late promoter (AdMLP)) and polyoma. For further description of viral regulatory elements, and sequences thereof, see *e.g.*, U.S. Patent No. 5,168,062 by Stinski, U.S. Patent No. 4,510,245 by Bell *et al.* and U.S. Patent No. 4,968,615 by Schaffner *et al.*, U.S. Patent No. 5,464,758 by Bujard *et al.* and U.S. Patent No. 5,654,168 by Bujard *et al.*

In addition to the antibody chain genes and regulatory sequences, the recombinant expression vectors of the invention may carry additional sequences, such as sequences that regulate replication of the vector in host cells (*e.g.*, origins of replication) and selectable marker genes. The selectable marker gene facilitates selection of host cells into which the vector has been introduced (see *e.g.*, U.S. Patents Nos. 4,399,216,

4,634,665 and 5,179,017, all by Axel *et al.*). For example, typically the selectable marker gene confers resistance to drugs, such as G418, hygromycin or methotrexate, on a host cell into which the vector has been introduced. Preferred selectable marker genes include the dihydrofolate reductase (DHFR) gene (for use in dhfr<sup>-</sup> host cells with  
5 methotrexate selection/amplification) and the *neo* gene (for G418 selection).

For expression of the light and heavy chains, the expression vector(s) encoding the heavy and light chains is transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or  
10 eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is theoretically possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells, and most preferably mammalian host cells, is the most preferred because such eukaryotic cells, and in particular mammalian cells, are more  
15 likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody. Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr<sup>-</sup> CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J.  
20 Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the  
25 host cells are grown. Antibodies can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce portions of intact antibodies, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be  
30 desirable to transfect a host cell with DNA encoding either the light chain or the heavy chain (but not both) of an antibody of this invention. Recombinant DNA technology may also be used to remove some or all of the DNA encoding either or both of the light

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and heavy chains that is not necessary for binding to hIL-12. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than hIL-12 by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Antibodies or antigen-binding portions thereof of the invention can be expressed in an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L.D. *et al.* (1992) *Nucl. Acids Res.* 20: 6287-6295). Plant cells can also be modified to create transgenic plants that express the antibody or antigen binding portion thereof, of the invention.

In view of the foregoing, another aspect of the invention pertains to nucleic acid, vector and host cell compositions that can be used for recombinant expression of the antibodies and antibody portions of the invention. Preferably, the invention features isolated nucleic acids that encode CDRs of J695, or the full heavy and/or light chain variable region of J695. Accordingly, in one embodiment, the invention features an isolated nucleic acid encoding an antibody heavy chain variable region that encodes the J695 heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 25.

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Preferably, the nucleic acid encoding the antibody heavy chain variable region further encodes a J695 heavy chain CDR2 which comprises the amino acid sequence of SEQ ID NO: 27. More preferably, the nucleic acid encoding the antibody heavy chain variable region further encodes a J695 heavy chain CDR1 which comprises the amino acid sequence of SEQ ID NO: 29. Even more preferably, the isolated nucleic acid encodes an antibody heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 31 (the full VH region of J695).

In other embodiments, the invention features an isolated nucleic acid encoding an antibody light chain variable region that encodes the J695 light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 26. Preferably, the nucleic acid encoding the antibody light chain variable region further encodes a J695 light chain CDR2 which comprises the amino acid sequence of SEQ ID NO: 28. More preferably, the nucleic acid encoding the antibody light chain variable region further encodes a J695 light chain CDR1 which comprises the amino acid sequence of SEQ ID NO: 30. Even more preferably, the isolated nucleic acid encodes an antibody light chain variable region comprising the amino acid sequence of SEQ ID NO: 32 (the full VL region of J695).

The invention also provides recombinant expression vectors encoding both an antibody heavy chain and an antibody light chain. For example, in one embodiment, the invention provides a recombinant expression vector encoding:

- a) an antibody heavy chain having a variable region comprising the amino acid sequence of SEQ ID NO: 31; and
- b) an antibody light chain having a variable region comprising the amino acid sequence of SEQ ID NO: 32.

The invention also provides host cells into which one or more of the recombinant expression vectors of the invention have been introduced. Preferably, the host cell is a mammalian host cell, more preferably the host cell is a CHO cell, an NS0 cell or a COS cell. Still further the invention provides a method of synthesizing a recombinant human antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant human antibody of the invention is synthesized. The method can further comprise isolating the recombinant human antibody from the culture medium.



## VI. Pharmaceutical Compositions and Pharmaceutical Administration

The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

The antibodies and antibody-portions of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody-portions will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05%

polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants.

In a preferred embodiment, the pharmaceutical composition includes the antibody at a dosage of about 0.01 mg/kg - 10 mg/kg. More preferred dosages of the antibody include 1 mg/kg administered every other week, or 0.3 mg/kg administered weekly.

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (*e.g.*, intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (*i.e.*, antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought

about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies and antibody-portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, e.g., *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which IL-12 activity is detrimental. For example, an anti-hIL-12 antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (e.g., antibodies that bind other cytokines or that bind cell surface

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molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies. It will be appreciated by the skilled practitioner that when the antibodies of the invention are used as part of a combination therapy, a lower dosage of antibody may be desirable than when the antibody alone is administered to a subject (e.g., a synergistic therapeutic effect may be achieved through the use of combination therapy which, in turn, permits use of a lower dose of the antibody to achieve the desired therapeutic effect).

Interleukin 12 plays a critical role in the pathology associated with a variety of diseases involving immune and inflammatory elements. These diseases include, but are not limited to, rheumatoid arthritis, osteoarthritis, juvenile chronic arthritis, Lyme arthritis, psoriatic arthritis, reactive arthritis, spondyloarthropathy, systemic lupus erythematosus, Crohn's disease, ulcerative colitis, inflammatory bowel disease, insulin dependent diabetes mellitus, thyroiditis, asthma, allergic diseases, psoriasis, dermatitis scleroderma, atopic dermatitis, graft versus host disease, organ transplant rejection, acute or chronic immune disease associated with organ transplantation, sarcoidosis, atherosclerosis, disseminated intravascular coagulation, Kawasaki's disease, Grave's disease, nephrotic syndrome, chronic fatigue syndrome, Wegener's granulomatosis, Henoch-Schoenlein purpura, microscopic vasculitis of the kidneys, chronic active hepatitis, uveitis, septic shock, toxic shock syndrome, sepsis syndrome, cachexia, infectious diseases, parasitic diseases, acquired immunodeficiency syndrome, acute transverse myelitis, Huntington's chorea, Parkinson's disease, Alzheimer's disease, stroke, primary biliary cirrhosis, hemolytic anemia, malignancies, heart failure, myocardial infarction, Addison's disease, sporadic, polyglandular deficiency type I and polyglandular deficiency type II, Schmidt's syndrome, adult (acute) respiratory distress syndrome, alopecia, alopecia areata, seronegative arthropathy, arthropathy, Reiter's disease, psoriatic arthropathy, ulcerative colitic arthropathy, enteropathic synovitis, chlamydia, yersinia and salmonella associated arthropathy, spondyloarthropathy, atheromatous disease/arteriosclerosis, atopic allergy, autoimmune bullous disease, pemphigus vulgaris, pemphigus foliaceus, pemphigoid, linear IgA disease, autoimmune

haemolytic anaemia. Coombs positive haemolytic anaemia, acquired pernicious anaemia, juvenile pernicious anaemia. myalgic encephalitis/Royal Free Disease, chronic mucocutaneous candidiasis, giant cell arteritis, primary sclerosing hepatitis, cryptogenic autoimmune hepatitis. Acquired Immunodeficiency Disease Syndrome, Acquired

5 Immunodeficiency Related Diseases, Hepatitis C, common varied immunodeficiency (common variable hypogammaglobulinaemia), dilated cardiomyopathy, female infertility, ovarian failure, premature ovarian failure. fibrotic lung disease, cryptogenic fibrosing alveolitis, post-inflammatory interstitial lung disease, interstitial pneumonitis, connective tissue disease associated interstitial lung disease. mixed connective tissue

10 disease associated lung disease, systemic sclerosis associated interstitial lung disease, rheumatoid arthritis associated interstitial lung disease, systemic lupus erythematosus associated lung disease, dermatomyositis/polymyositis associated lung disease, Sjögren's disease associated lung disease, ankylosing spondylitis associated lung disease, vasculitic diffuse lung disease, haemosiderosis associated lung disease, drug-induced

15 interstitial lung disease, radiation fibrosis, bronchiolitis obliterans, chronic eosinophilic pneumonia, lymphocytic infiltrative lung disease. postinfectious interstitial lung disease, gouty arthritis, autoimmune hepatitis, type-1 autoimmune hepatitis (classical autoimmune or lupoid hepatitis), type-2 autoimmune hepatitis (anti-LKM antibody hepatitis), autoimmune mediated hypoglycemia, type B insulin resistance with

20 acanthosis nigricans, hypoparathyroidism, acute immune disease associated with organ transplantation, chronic immune disease associated with organ transplantation, osteoarthritis, primary sclerosing cholangitis, idiopathic leucopenia, autoimmune neutropenia, renal disease NOS, glomerulonephritides, microscopic vasculitis of the kidneys, lyme disease, discoid lupus erythematosus, male infertility idiopathic or NOS,

25 sperm autoimmunity, multiple sclerosis (all subtypes), insulin-dependent diabetes mellitus, sympathetic ophthalmia, pulmonary hypertension secondary to connective tissue disease, Goodpasture's syndrome, pulmonary manifestation of polyarteritis nodosa, acute rheumatic fever, rheumatoid spondylitis, Still's disease, systemic sclerosis, Takayasu's disease/arteritis, autoimmune thrombocytopenia, idiopathic

30 thrombocytopenia, autoimmune thyroid disease, hyperthyroidism, goitrous autoimmune hypothyroidism (Hashimoto's disease), atrophic autoimmune hypothyroidism, primary myxoedema, phacogenic uveitis, primary vasculitis and vitiligo. The human antibodies,

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and antibody portions of the invention can be used to treat autoimmune diseases, in particular those associated with inflammation, including, rheumatoid spondylitis, allergy, autoimmune diabetes, autoimmune uveitis.

Preferably, the antibodies of the invention or antigen-binding portions thereof, are used to treat rheumatoid arthritis, Crohn's disease, multiple sclerosis, insulin dependent diabetes mellitus and psoriasis, as described in more detail in section VII.

A human antibody, or antibody portion, of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune and inflammatory diseases.

Antibodies of the invention, or antigen binding portions thereof can be used alone or in combination to treat such diseases. It should be understood that the antibodies of the invention or antigen binding portion thereof can be used alone or in combination with an additional agent, e.g., a therapeutic agent, said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent art-recognized as being useful to treat the disease or condition being treated by the antibody of the present invention. The additional agent also can be an agent which imparts a beneficial attribute to the therapeutic composition e.g., an agent which effects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations which are part of this invention can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

Preferred combinations are non-steroidal anti-inflammatory drug(s) also referred to as NSAIDS which include drugs like ibuprofen. Other preferred combinations are corticosteroids including prednisolone; the well known side-effects of steroid use can be reduced or even eliminated by tapering the steroid dose required when treating patients in combination with the anti-IL-12 antibodies of this invention. Non-limiting examples of therapeutic agents for rheumatoid arthritis with which an antibody, or antibody

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portion, of the invention can be combined include the following: cytokine suppressive anti-inflammatory drug(s) (CSAIDs); antibodies to or antagonists of other human cytokines or growth factors. for example. TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or  
5 antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4, CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80 (B7.1), CD86 (B7.2), CD90, or their ligands including CD154 (gp39 or CD40L).

Preferred combinations of therapeutic agents may interfere at different points in the autoimmune and subsequent inflammatory cascade; preferred examples include TNF  
10 antagonists like chimeric, humanized or human TNF antibodies, D2E7, (U.S. application serial number 08/599,226 filed February 9, 1996), cA2 (Remicade™), CDP 571, anti-TNF antibody fragments (*e.g.*, CDP870), and soluble p55 or p75 TNF receptors, derivatives thereof, (p75TNFR1gG (Enbrel™) or p55TNFR1gG (Lenercept), soluble IL-13 receptor (sIL-13), and also TNF $\alpha$  converting enzyme (TACE) inhibitors; similarly  
15 IL-1 inhibitors (*e.g.*, Interleukin-1-converting enzyme inhibitors, such as Vx740, or IL-1RA etc.) may be effective for the same reason. Other preferred combinations include Interleukin 11, anti-P7s and p-selectin glycoprotein ligand (PSGL). Yet another preferred combination are other key players of the autoimmune response which may act parallel to, dependent on or in concert with IL-12 function; especially preferred are IL-  
20 18 antagonists including IL-18 antibodies or soluble IL-18 receptors, or IL-18 binding proteins. It has been shown that IL-12 and IL-18 have overlapping but distinct functions and a combination of antagonists to both may be most effective. Yet another preferred combination are non-depleting anti-CD4 inhibitors. Yet other preferred combinations include antagonists of the co-stimulatory pathway CD80 (B7.1) or CD86 (B7.2)  
25 including antibodies, soluble receptors or antagonistic ligands.

The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, 6-MP, azathioprine sulphasalazine, mesalazine, olsalazine chloroquine/hydroxychloroquine, pencillamine, aurothiomalate (intramuscular and oral), azathioprine, cochlincine, corticosteroids (oral, inhaled and local  
30 injection), beta-2 adrenoreceptor agonists (salbutamol, terbutaline, salmeteral), xanthines (theophylline, aminophylline), cromoglycate, nedocromil, ketotifen, ipratropium and oxitropium, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide,

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NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF $\alpha$  or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors),

5 IL-1 $\beta$  converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein ligand (PSGL), TNF $\alpha$  converting enzyme (TACE) inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors and the derivatives

10 p75TNFRlgG (Enbrel<sup>TM</sup>) and p55TNFRlgG (Lencrcept), sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and TGF $\beta$ ). Preferred combinations include methotrexate or leflunomide and in moderate or severe rheumatoid arthritis cases, cyclosporine.

Non-limiting examples of therapeutic agents for inflammatory bowel disease

15 with which an antibody, or antibody portion, of the invention can be combined include the following: budenoside; epidermal growth factor; corticosteroids; cyclosporin, sulfasalazine; aminosalicylates; 6-mercaptopurine; azathioprine; metronidazole; lipoxigenase inhibitors; mesalamine; olsalazine; balsalazide; antioxidants; thromboxane inhibitors; IL-1 receptor antagonists; anti-IL-1 $\beta$  monoclonal antibodies; anti-IL-6

20 monoclonal antibodies; growth factors; elastase inhibitors; pyridinyl-imidazole compounds; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4,

25 CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD90 or their ligands. The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporin, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement

30 inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF $\alpha$  or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 $\beta$  converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein



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ligand (PSGL), TNF $\alpha$  converting enzyme inhibitors, T-cell signalling inhibitors such as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptapurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-11, IL-13 and TGF $\beta$ ).

Preferred examples of therapeutic agents for Crohn's disease in which an antibody or an antigen binding portion can be combined include the following: TNF antagonists, for example, anti-TNF antibodies, D2E7 (U.S. application serial number 08/599,226, filed February 9, 1996), cA2 (Remicade<sup>TM</sup>), CDP 571, anti-TNF antibody fragments (e.g., CDP870), TNFR-Ig constructs (p75TNFRIgG (Enbrel<sup>TM</sup>) and p55TNFRIgG (Lenercept)), anti-P7s, p-selectin glycoprotein ligand (PSGL), soluble IL-13 receptor (sIL-13), and PDE4 inhibitors. Antibodies of the invention or antigen binding portions thereof, can be combined with corticosteroids, for example, budenoside and dexamethasone. Antibodies of the invention or antigen binding portions thereof, may also be combined with agents such as sulfasalazine, 5-aminosalicylic acid and olsalazine, and agents which interfere with synthesis or action of proinflammatory cytokines such as IL-1, for example, IL-1 $\beta$  converting enzyme inhibitors (e.g., Vx740) and IL-1ra. Antibodies of the invention or antigen binding portion thereof may also be used with T cell signaling inhibitors, for example, tyrosine kinase inhibitors 6-mercaptapurines. Antibodies of the invention or antigen binding portions thereof, can be combined with IL-11.

Non-limiting examples of therapeutic agents for multiple sclerosis with which an antibody, or antibody portion, of the invention can be combined include the following: corticosteroids; prednisolone; methylprednisolone; azathioprine; cyclophosphamide; cyclosporine; methotrexate; 4-aminopyridine; tizanidine; interferon- $\beta$ 1a (Avonex; Biogen); interferon- $\beta$ 1b (Betaseron; Chiron/Berlex); Copolymer 1 (Cop-1; Copaxone; Teva Pharmaceutical Industries, Inc.); hyperbaric oxygen; intravenous immunoglobulin; clabribine; antibodies to or antagonists of other human cytokines or growth factors, for example, TNF, LT, IL-1, IL-2, IL-6, IL-7, IL-8, IL-15, IL-16, IL-18, EMAP-II, GM-CSF, FGF, and PDGF. Antibodies of the invention, or antigen binding portions thereof, can be combined with antibodies to cell surface molecules such as CD2, CD3, CD4,

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CD8, CD25, CD28, CD30, CD40, CD45, CD69, CD80, CD86, CD90 or their ligands.

The antibodies of the invention, or antigen binding portions thereof, may also be combined with agents, such as methotrexate, cyclosporine, FK506, rapamycin, mycophenolate mofetil, leflunomide, NSAIDs, for example, ibuprofen, corticosteroids  
5 such as prednisolone, phosphodiesterase inhibitors, adenosine agonists, antithrombotic agents, complement inhibitors, adrenergic agents, agents which interfere with signalling by proinflammatory cytokines such as TNF $\alpha$  or IL-1 (e.g. IRAK, NIK, IKK, p38 or MAP kinase inhibitors), IL-1 $\beta$  converting enzyme inhibitors (e.g., Vx740), anti-P7s, p-selectin glycoprotein ligand (PSGL), TACE inhibitors, T-cell signalling inhibitors such  
10 as kinase inhibitors, metalloproteinase inhibitors, sulfasalazine, azathioprine, 6-mercaptopurines, angiotensin converting enzyme inhibitors, soluble cytokine receptors and derivatives thereof (e.g. soluble p55 or p75 TNF receptors, sIL-1RI, sIL-1RII, sIL-6R, soluble IL-13 receptor (sIL-13)) and antiinflammatory cytokines (e.g. IL-4, IL-10, IL-13 and TGF $\beta$ ).

15 Preferred examples of therapeutic agents for multiple sclerosis in which the antibody or antigen binding portion thereof can be combined to include interferon- $\beta$ , for example, IFN $\beta$ 1a and IFN $\beta$ 1b; copaxone, corticosteroids, IL-1 inhibitors, TNF inhibitors, and antibodies to CD40 ligand and CD80.

The pharmaceutical compositions of the invention may include a "therapeutically  
20 effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may vary according to factors such as the disease state, age, sex, and weight of the individual, and  
25 the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired  
30 prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

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Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.01-20 mg/kg, more preferably 1-10 mg/kg, even more preferably 0.3-1 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

## VII. Uses of the Antibodies of the Invention

Given their ability to bind to hIL-12, the anti-hIL-12 antibodies, or portions thereof, of the invention can be used to detect hIL-12 (*e.g.*, in a biological sample, such as serum or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention provides a method for detecting hIL-12 in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion)

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bound to hIL-12 or unbound antibody (or antibody portion), to thereby detect hIL-12 in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase,  $\beta$ -galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include  $^{125}\text{I}$ ,  $^{131}\text{I}$ ,  $^{35}\text{S}$  or  $^3\text{H}$ .

Alternative to labeling the antibody, hIL-12 can be assayed in biological fluids by a competition immunoassay utilizing rhIL-12 standards labeled with a detectable substance and an unlabeled anti-hIL-12 antibody. In this assay, the biological sample, the labeled rhIL-12 standards and the anti-hIL-12 antibody are combined and the amount of labeled rhIL-12 standard bound to the unlabeled antibody is determined. The amount of hIL-12 in the biological sample is inversely proportional to the amount of labeled rhIL-12 standard bound to the anti-hIL-12 antibody.

The Y61 and J695 antibodies of the invention can also be used to detect IL-12 from species other than humans, in particular IL-12 from primates. For example, Y61 can be used to detect IL-12 in the cynomolgus monkey and the rhesus monkey. J695 can be used to detect IL-12 in the cynomolgus monkey, rhesus monkey, and baboon. However, neither antibody cross reacts with mouse or rat IL-12 (see Example 3, subsection F).

The antibodies and antibody portions of the invention are capable of neutralizing hIL-12 activity *in vitro* (see Example 3) and *in vivo* (see Example 4). Accordingly, the antibodies and antibody portions of the invention can be used to inhibit IL-12 activity, *e.g.*, in a cell culture containing hIL-12, in human subjects or in other mammalian subjects having IL-12 with which an antibody of the invention cross-reacts (*e.g.* primates such as baboon, cynomolgus and rhesus). In a preferred embodiment, the invention provides an isolated human antibody, or antigen-binding portion thereof, that neutralizes the activity of human IL-12, and at least one additional primate IL-12

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selected from the group consisting of baboon IL-12, marmoset IL-12, chimpanzee IL-12, cynomolgus IL-12 and rhesus IL-12, but which does not neutralize the activity of the mouse IL-12. Preferably, the IL-12 is human IL-12. For example, in a cell culture containing, or suspected of containing hIL-12, an antibody or antibody portion of the invention can be added to the culture medium to inhibit hIL-12 activity in the culture.

In another embodiment, the invention provides a method for inhibiting IL-12 activity in a subject suffering from a disorder in which IL-12 activity is detrimental. IL-12 has been implicated in the pathophysiology of a wide variety of disorders (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996; Morita *et al.* (1998) *Arthritis and Rheumatism*. 41: 306-314; Bucht *et al.*, (1996) *Clin. Exp. Immunol.* 103: 347-367; Fais *et al.* (1994) *J. Interferon Res.* 14:235-238; Parronchi *et al.*, (1997) *Am. J. Path.* 150:823-832; Monteleone *et al.*, (1997) *Gastroenterology*. 112:1169-1178, and Berrebi *et al.*, (1998) *Am. J. Path.* 152:667-672; Parronchi *et al.* (1997) *Am. J. Path.* 150:823-832). The invention provides methods for inhibiting IL-12 activity in a subject suffering from such a disorder, which method comprises administering to the subject an antibody or antibody portion of the invention such that IL-12 activity in the subject is inhibited. Preferably, the IL-12 is human IL-12 and the subject is a human subject. Alternatively, the subject can be a mammal expressing a IL-12 with which an antibody of the invention cross-reacts. Still further the subject can be a mammal into which has been introduced hIL-12 (*e.g.*, by administration of hIL-12 or by expression of an hIL-12 transgene). An antibody of the invention can be administered to a human subject for therapeutic purposes (discussed further below). Moreover, an antibody of the invention can be administered to a non-human mammal expressing a IL-12 with which the antibody cross-reacts for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (*e.g.*, testing of dosages and time courses of administration).

As used herein, the phrase "a disorder in which IL-12 activity is detrimental" is intended to include diseases and other disorders in which the presence of IL-12 in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which IL-12 activity is detrimental is a disorder in which inhibition of IL-12 activity is expected to alleviate the

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symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of IL-12 in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of IL-12 in serum, plasma, synovial fluid, *etc.* of the subject), which can be detected, for example, using an anti-IL-12 antibody as described above. There are numerous examples of disorders in which IL-12 activity is detrimental. In one embodiment, the antibodies or antigen binding portions thereof, can be used in therapy to treat the diseases or disorders described herein. In another embodiment, the antibodies or antigen binding portions thereof, can be used for the manufacture of a medicine for treating the diseases or disorders described herein. The use of the antibodies and antibody portions of the invention in the treatment of a few non-limiting specific disorders is discussed further below:

*A. Rheumatoid Arthritis:*

Interleukin-12 has been implicated in playing a role in inflammatory diseases such as rheumatoid arthritis. Inducible IL-12p40 message has been detected in synovia from rheumatoid arthritis patients and IL-12 has been shown to be present in the synovial fluids from patients with rheumatoid arthritis (see *e.g.*, Morita *et al.*, (1998) *Arthritis and Rheumatism* 41: 306-314). IL-12 positive cells have been found to be present in the sublining layer of the rheumatoid arthritis synovium. The human antibodies, and antibody portions of the invention can be used to treat, for example, rheumatoid arthritis, juvenile rheumatoid arthritis, Lyme arthritis, rheumatoid spondylitis, osteoarthritis and gouty arthritis. Typically, the antibody, or antibody portion, is administered systemically, although for certain disorders, local administration of the antibody or antibody portion may be beneficial. An antibody, or antibody portion, of the invention also can be administered with one or more additional therapeutic agents useful in the treatment of autoimmune diseases.

In the collagen induced arthritis (CIA) murine model for rheumatoid arthritis, treatment of mice with an anti-IL-12 mAb (rat anti-mouse IL-12 monoclonal antibody, C17.15) prior to arthritis profoundly suppressed the onset, and reduced the incidence and severity of disease. Treatment with the anti-IL-12 mAb early after onset of arthritis

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reduced severity, but later treatment of the mice with the anti-IL-12 mAb after the onset of disease had minimal effect on disease severity.

#### B. Crohn's Disease

5 Interleukin-12 also plays a role in the inflammatory bowel disease, Crohn's disease. Increased expression of IFN- $\gamma$  and IL-12 occurs in the intestinal mucosa of patients with Crohn's disease (see e.g., Fais *et al.*, (1994) *J. Interferon Res.* 14: 235-238; Parronchi *et al.*, (1997) *Amer. J. Pathol.* 150: 823-832; Monteleone *et al.*, (1997) *Gastroenterology* 112: 1169-1178; Berrebi *et al.*, (1998) *Amer. J. Pathol.* 152:  
10 667-672). Anti-IL-12 antibodies have been shown to suppress disease in mouse models of colitis, e.g., TNBS induced colitis IL-2 knockout mice, and recently in IL-10 knock-out mice. Accordingly, the antibodies, and antibody portions, of the invention, can be used in the treatment of inflammatory bowel diseases.

#### 15 C. Multiple Sclerosis

Interleukin-12 has been implicated as a key mediator of multiple sclerosis. Expression of the inducible IL-12 p40 message or IL-12 itself can be demonstrated in lesions of patients with multiple sclerosis (Windhagen *et al.*, (1995) *J. Exp. Med.* 182: 1985-1996, Drulovic *et al.*, (1997) *J. Neurol. Sci.* 147: 145-150).  
20 Chronic progressive patients with multiple sclerosis have elevated circulating levels of IL-12. Investigations with T-cells and antigen presenting cells (APCs) from patients with multiple sclerosis revealed a self-perpetuating series of immune interactions as the basis of progressive multiple sclerosis leading to a Th1-type immune response. Increased secretion of IFN- $\gamma$  from the T cells led to increased IL-12 production by  
25 APCs, which perpetuated the cycle leading to a chronic state of a Th1-type immune activation and disease (Balashov *et al.*, (1997) *Proc. Natl. Acad. Sci.* 94: 599-603). The role of IL-12 in multiple sclerosis has been investigated using mouse and rat experimental allergic encephalomyelitis (EAE) models of multiple sclerosis. In a relapsing-remitting EAE model of multiple sclerosis in mice, pretreatment with anti-IL-  
30 12 mAb delayed paralysis and reduced clinical scores. Treatment with anti-IL-12 mAb at the peak of paralysis or during the subsequent remission period reduced clinical

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scores. Accordingly, the antibodies or antigen binding portions thereof of the invention may serve to alleviate symptoms associated with multiple sclerosis in humans.

*D. Insulin-Dependent Diabetes Mellitus*

5 Interleukin-12 has been implicated as an important mediator of insulin-dependent diabetes mellitus (IDDM). IDDM was induced in NOD mice by administration of IL-12, and anti-IL-12 antibodies were protective in an adoptive transfer model of IDDM. Early onset IDDM patients often experience a so-called "honeymoon period" during which some residual islet cell function is maintained. These  
10 residual islet cells produce insulin and regulate blood glucose levels better than administered insulin. Treatment of these early onset patients with an anti-IL-12 antibody may prevent further destruction of islet cells, thereby maintaining an endogenous source of insulin.

15 *E. Psoriasis*

Interleukin-12 has been implicated as a key mediator in psoriasis. Psoriasis involves acute and chronic skin lesions that are associated with a TH1-type cytokine expression profile. (Hamid et al. (1996) J. Allergy Clin. Immunol. 1:225-231; Turka et al. (1995) Mol. Med. 1:690-699). IL-12 p35 and p40 mRNAs were detected in  
20 diseased human skin samples. Accordingly, the antibodies or antigen binding portions thereof of the invention may serve to alleviate chronic skin disorders such psoriasis.

The present invention is further illustrated by the following examples which should not be construed as limiting in any way. The contents of all cited references, including literature references, issued patents, and published patent applications, as cited  
25 throughout this application are hereby expressly incorporated by reference. It should further be understood that the contents of all the tables attached hereto (see Appendix A) are incorporated by reference.



APPENDIX A

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Table 1 VH3 Family Germline Amino Acid Sequences Numbering according to Kabat (Joe9\_VH included for comparison)

SEQ ID NO:	germline	CDR H1	CDR H2
594	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DIYMD	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
595	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
596	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
597	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
598	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
599	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
600	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
601	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
602	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
603	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
604	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
605	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
606	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
607	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
608	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
609	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
610	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
611	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
612	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
613	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
614	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
615	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
616	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
617	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
618	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
619	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
620	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
621	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
622	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
623	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
624	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
625	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
626	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
627	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
628	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
629	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
630	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
631	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
632	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
633	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
634	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
635	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
636	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
637	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
638	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR
639	EVOLVESGGGLVQPGGSLRLSCAASGFTFS	DHYHS	RTISRDSSKNTLYLQMSLRAEDTAVYTCAR

## APPENDIX A

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Table 1  
VH3 Family Germline Amino Acid Sequences  
Numbering according to Kabat  
(Joe9 VH included for comparison)

SEQ ID	germline	CDR H1	CDR H2
MO:	VH		
640	B1	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
641	B13	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
642	B18	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
643	B26	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
644	B28E	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
645	B29E	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
646	B29H	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
647	B30	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
648	B32H	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
649	cos-3	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
650	dp-49	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
651	dp-50	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
652	P6	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
653	P9E	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
654	v3-30	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
655	v3-33	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
656	dp-31	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
657	dp-77	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
658	HKG4	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
659	v3-21	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
660	v3-48	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
661	dp-52	EDQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
662	cos-6	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
663	dp-53	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
664	dp-54	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
665	dp-87	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
666	VH3-11	EVQLVSGGGLVQPGGSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG
667	JOE9 VH	QVQLVSGGCVVQPGKSLRLSCAASGFTFS	WVRQAPGKGLEHVA VI...SYDGSNKYYADSVKG

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Table 1 Vλ1 Family Germline Amino Acid Sequences  
Numbering according to Kabat.  
(Joe9 VL included for comparison)

SEQ ID	gene*	VL	CDR L1										CDR L2										CDR L3																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																													
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\*Williams, JMB, 1996, 264, 220-232

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
Joe9 wt	77	SGSYDY	110	QSYDSSLRGSRV	1.00E-01	1.50E-06	1.00E-06	
Joe9 wt IgG1	77	SGSYDY	110	QSYDSSLRGSRV			5.00E-07	
70-1	78	HGSHDN	110	Joe9 wt	1.34 e-2		2.00E-07	
70-1 IgG1	78	HGSHDN	110	Joe9 wt			2.00E-07	
70-2	79	HGSHDN	110	Joe9 wt	3.30E-02		3-5.0E-7	
70-7	80	RRRSNY	110	Joe9 wt	1.29E-01		3-5.0E-7	
70-13	81	SGSIDY	110	Joe9 wt	7.20E-02		3-5.0E-7	
78-34	77	wt	111	QSYDRGFTGSRV	1.64 e-2	2.00E-07	6.00E-07	
78-25	77	wt	112	QSYDSSLRGSRV	5.00E-02			
78-28	77	wt	112	QSYDSSLRGSRV	4.66E-02			
78-35	77	wt	113	QSYDSSLTGSRV	4.99E-02	4.00E-07		
79-1	77	wt	114	QSYDSSLMGSRV		2.00E-07	6.00E-07	
101-14	79	70-2	111	78-34	7.52E-03			
101-9	79	70-2	113	78-35	8.54E-03			
101-19	81	70-13	111	78-34	4.56E-02			
101-8	81	70-13	111	78-34	1.01E-02			
101-4	81	70-13	113	78-35	9.76E-03			
101-5	81	70-13	113	78-35	4.45E-02			
101-11 (12)	78	70-1	111	78-34	4.5 e-3		3.00E-08	
101-11 IgG1	78	70-1	111	78-34		1.60E-09		
26-1 (2,3)	78	70-1	114	79-1	7.4 e-3		6.00E-08	
136-9	82	HGSHDD	115	QTYDISEGSRV	3.20E-03			
136-10	82	HGSHDD	116	QSYDRGFTGSRV	1.40E-03	2.00E-09		
136-14	83	HGSHDN	117	QTYDRGFTGSRV	1.10E-03	3.00E-10	1.00E-07	
136-15	83	HGSHDN	118	QTYDKGFTGSSV	7.4 e-4	1.00E-10	2.00E-09	
136-15 germline	83	HGSHDN	118	QTYDKGFTGSSV	4.60E-04		6.00E-09	
136-16	83	HGSHDN	119	QSYDRRFTGSRV	6.10E-04	3.00E-10	5.00E-09	
136-17	83	HGSHDN	120	QSYDWNFTGSRV	2.90E-05	2.00E-09	7.00E-09	
136-18	83	HGSHDN	121	QSYDRGFTGSRV	1.10E-03	8.00E-10		
136-21	83	HGSHDN	122	QSYDNGFTGSRV	4.20E-04	2.00E-09		
136-24	83	HGSHDN	123	QSYDNVATASKV	8.90E-04	1.00E-09		

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
101-11	84	TT HGSHDN HGQG	124	QSYDRGFTGSRV	4.5x10 <sup>-3</sup>	2x10 <sup>-9</sup>	2.00E-08	
136-15M1	85	AK .....	124	QSYDRGFTGSRV		4.00E-10		
149-4	86	.. .....	124	.....S..	1.37x10 <sup>-3</sup>	8x10 <sup>-11</sup>	3.00E-09	
149-5	87	.. .....	125	QSYDSSLWGTRV	1.02x10 <sup>-3</sup>	1.2x10 <sup>-10</sup>	3.00E-09	
149-6	84	.. .....	124	.....D.....	2.73x10 <sup>-3</sup>	6x10 <sup>-10</sup>	2.00E-09	
149-7	84	.. .....	126	.....D.....	1.13x10 <sup>-3</sup>	9x10 <sup>-10</sup>	3.00E-09	
149-8	88	K. ....			2.33x10 <sup>-3</sup>	3x10 <sup>-9</sup>		
149-9	89	K. ....H.	127	...E.....M.	3.54x10 <sup>-3</sup>	1.8x10 <sup>-10</sup>		
149-11	90	.. .....	128	...N....A..	1.43x10 <sup>-2</sup>	2x10 <sup>-10</sup>	4.00E-09	
149-12	84	.. .....			3.73x10 <sup>-3</sup>	neutralising		
149-13	84	.. .....			2.22x10 <sup>-3</sup>	5x10 <sup>-10</sup>		
149-14	91	.. ..R..N. ....				1.5x10 <sup>-10</sup>	6.00E-09	
156-1	92	TT HGSHDN	124	QSYDRGFTGSRV				
156-2	93	.. .....	126	.....D.....	5.00E-03			
156-3	93	.. .....	129	.....R.....				
156-4	93	.. .....	128	...N....A..	9.00E-03			
156-5	93	.. .....	121	...E.....SM.				
156-6	92	.. .....	130	.T..K.....S.				
156-7	92	.. .....	126	.....D.....	3.00E-03			
156-8	92	.. .....	129	.....R.....				
156-9	92	.. .....	128	...N....A..				
156-10	92	.. .....	127	...E.....SM.				
156-11	94	.K .....	130	.T..K.....S.				
156-12	94	.K .....	126	.....D.....				
156-13	94	.K .....	129	.....R.....				
156-14	94	.K .....	128	...N....A..				
156-15	94	.K .....	127	...E.....SM.				
156-16	93	.. .....	130	.T..K.....S.				
156-17	92	.. .....	124	.....D.....				
156-18	93	.. .....	125	...SSLW.T..	6.00E-03			
			125	...SSLW.T..				

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
	92	TT HGSHDN	124	QSYDRGETGSRV				
103-1	95	.. Q.R...	124	.....	2.9x10 <sup>-3</sup>			
103-2	96	K. R.R...	130	.T..K.....S.	7.3x10 <sup>-4</sup>	7.00E-11	1.00E-09	
103-3	97	.. .....K	124	.....	2.5x10 <sup>-3</sup>			
103-6			131	.....D...T..	4.5x10 <sup>-4</sup>			
103-7	98	.. .....D	131	.....D...T..	3.7x10 <sup>-4</sup>	1.40E-10	1.00E-09	
103-8	99	K. ....	130	.T..K.....S.	3.3x10 <sup>-4</sup>	6.00E-11	1.50E-09	
103-14 & 9	100	KT HGSHDN	132	QSYDRGFTGSMV	6.7 e-4	4.00E-11	1.20E-09	
103-8 & 2	100	KT HGSHDN	133	QYDKGFTGSSV	5.3 e-4		1.50E-09	
103-4	101	TT HGSHDN	134	QSYDRGFTGARV	1.6 e-4	8.60E-11	9.00E-10	
103-152	101	TT HGSHDN	135	QSYERGFTGARV		8.60E-11		
	102	TT SGSDX	136	QSYDRGFTGSRVF				
170-1	102	.. ....	137	.....FK..	2.35E-03			
170-2	102	.. ....	138	.....VSAY..	8.80E-04			
170-3	102	.. ....	139	.....L.VTK..	1.11E-03			
170-4	102	.. ....	140	.....Y.A....	8.11E-04			
170-7	102	.. ....	141	.....K..	5.30E-04			
170-11	102	.. ....	142	.....L..F...	4.40E-04			
170-13	102	.. ....	143	.....YK..	1.59E-03			
170-15	102	.. ....	144	.....L..Y.L.	4.43E-03			
170-19	103	.. H..H.N	145	.....DYK..	1.00E-03			
170-21	104	.. H..Q.N	146	.....P.L.	3.89E-03			
170-22	102	.. ....	147	.....L.....	5.60E-04			
170-23	103	.. H..H.N	148	.....A..W	1.00E-03	2.00E-10		
170-24	104	.. H..Q.N	149	.....Y...	2.80E-04	5.00E-10		
170-35	105	A. H..Q.N	136	.....	1.00E-05			
170-38			150	.....P...	2.10E-04			
170-39			151	.....M.S....	2.79E-03			
170-36	83	HGSHDN	152	QSYDRDSTGSRVF	4.00E-04	2.00E-10		
170-25	106	HGSQDT	153	QSYDSSLRGSRVF	5.00E-04	5.00E-11		

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**Table 2**

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
	106	SGSYDY	136	QBYDRGFTGSRVF				
73-B1	107	SGSYDY	154	H...SD.....	3.25E-03	>1E-8		
73-B2	107	SGSYDY	155	H.SES.....	2.07E-03			
73-B6	107	SGSYDY	156	H...NR.....	2.51E-03	>1E-8		
73-C1	107	SGSYDY	157	H...SR.....	2.71E-03	>1E-8		
73-C2	107	SGSYDY	158	....SE.....	3.79E-03			
73-C6	107	SGSYDY	159	....T.....	3.96E-03			
73-D1	107	SGSYDY	160	H...S.....	3.99E-03			
73-D2	107	SGSYDY	161	....T.....	3.56E-03			
73-D4	107	SGSYDY	162	H...TK.....	5.36E-03			
73-D5	107	SGSYDY	163	H.S.S.....	3.57E-03			
73-E3	107	SGSYDY	164	....SD.....	4.98E-03			
73-E6	107	SGSYDY	165	H..ES.....	4.17E-03			
73-F3	107	SGSYDY	166	....APWS.....	7.08E-03			
73-F5	107	SGSYDY	167	...DSD....K..	3.74E-03			
73-G2	107	SGSYDY	168	HTN.S.....	3.98E-03			
73-G3	107	SGSYDY	169	H...TR.....	3.50E-03			
73-G4	107	SGSYDY	170	....MR.....	6.58E-03			
73-G5	107	SGSYDY	171	H.S.SDS.....	6.01E-03			
73-G6	107	SGSYDY	172	...NTD.....	6.30E-03			
73-H2	107	SGSYDY	173	....S.....	5.93E-03			
73-F6	107	SGSYDY	174	H...M.....	5.87E-03			
73-H3	107	SGSYDY	175	H...N.....	6.85E-03			
73-C5	107	SGSYDY	176	H.H..D.....	4.84E-03			
73-B7	108	HGSQDN	177	QSYDSSLRGSRV	2.50E-03	7.00E-09		

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
			136	QSYDRGETGSRVF				
M2 A2	83	HGSHDN	178	.....IH.....	4.00E-02			
M2 A4	83	HGSHDN	179	.....S..P.....	8.49E-03			
M2 A5	83	HGSHDN	180	.....I.S.....	4.01E-02			
M2 B1	83	HGSHDN	181	.....S.L.....	7.97E-03			
M2 B3	83	HGSHDN	182	.....I.M.....	4.60E-02			
M2 B4	83	HGSHDN	183	.....I.L.....	4.42E-02			
M2 B5	83	HGSHDN	184	.....S.V.....	8.38E-03			
M2 B6	83	HGSHDN	185	.....L.A.....	2.81E-02			
M2 C2	83	HGSHDN	181	.....S.L.....	4.85E-02			
M2 C3	83	HGSHDN	186	.....T.L.....	4.62E-02			
M2 C4	83	HGSHDN	181	.....S.L.....	8.16E-03			
M2 C5	83	HGSHDN	187	.....TAL.....	4.71E-02			
M2 D1	83	HGSHDN	188	.....IR.....	3.71E-02			
M2 D2	83	HGSHDN	189	.....IRS.....	3.85E-02			
M2 D3	83	HGSHDN	190	.....NRL.....	3.33E-02			
M2 D4	83	HGSHDN	191	...ETS.....	5.81E-02			
M2 D5	83	HGSHDN	192	....SSS.....	5.18E-02			
M2 D6	83	HGSHDN	193	....S...A....	5.01E-02			
M2 E1	83	HGSHDN	194	.T..K.....S..	5.32E-02			
M2 E2	83	HGSHDN	195	....N.....	4.77E-02			
M2 E6	83	HGSHDN	196	....T...K....	9.77E-03			
M2 F1	83	HGSHDN	197	....SDV.....	6.16E-02			
M2 H5	83	HGSHDN	198	....A.....	9.90E-03			



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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
A5	83	HGSHDN	124	QSYDRGEGTGSRY				
A12	83	HGSHDN	199	.....THPSML	1.12E-03			
A4	83	HGSHDN	200	.....TTPRPM	1.43E-03			
A6	83	HGSHDN	201	.....RNPALT	1.47E-03			
A10	83	HGSHDN	202	.....THPWLH	1.87E-03			
A11	83	HGSHDN	203	.....NSPATV	1.87E-03			
C2	83	HGSHDN	204	.....TFPSQ	2.07E-03			
A8	83	HGSHDN	205	.....LNPSAT	2.23E-03			
B8	83	HGSHDN	206	.....KSNKML	2.37E-03			
C6	83	HGSHDN	207	.....HTAHLV	2.40E-03			
A3	83	HGSHDN	208	.....QTPSIT	2.42E-03			
B11	83	HGSHDN	209	.....YPRNIL	2.51E-03			
B5	83	HGSHDN	210	.....ITPGLA	2.95E-03			
C10	83	HGSHDN	211	.....QPHAVL	3.04E-03			
C4	83	HGSHDN	212	.....NSPIPT	3.10E-03			
C3	83	HGSHDN	213	.....TPNNSF	3.23E-03			
B2	83	HGSHDN	214	....S.VDPGPY	3.34E-03			
A2	83	HGSHDN	215	.....RPRHAL	3.61E-03			
C5	83	HGSHDN	216	.....PYHPIR	3.80E-03			
A7	83	HGSHDN	217	.....PHTOPT	3.91E-03			
C9	83	HGSHDN	218	.....HNNFSP	3.95E-03			
B3	83	HGSHDN	219	.....PHTLPH	3.97E-03			
C8	83	HGSHDN	220	.....TPSYPT	4.12E-03			
B7	83	HGSHDN	221	....S.TSNLLP	5.36E-03			
A1	83	HGSHDN	222	.....DSNHDL	5.45E-03			
C7	83	HGSHDN	223	.....LPRLTH	5.66E-03			
C12	83	HGSHDN	224	.....IPTSYL	5.83E-03			
B10	83	HGSHDN	225	.....LRVQAP	5.85E-03			
B6	83	HGSHDN	226	.....LSDGSL	6.04E-03			
A9	83	HGSHDN	227	....S.SLRRL	7.58E-03			
B9	83	HGSHDN	228	.....PARTSP	7.98E-03			
			229	.....RAHPQ	8.66E-03			

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
			124	QBYDRGEGTSRY				
177-D7	83	HGSHDN	230	.....TQPABI	4.07E-04			
177-G6	83	HGSHDN	231	.....THPTMI	5.50E-04			
177-D9	83	HGSHDN	232	.....RIPABT	6.32E-04			
177-C6	83	HGSHDN	233	.....THPVPA	7.94E-04			
177-H5	83	HGSHDN	234	.....SBPIPA	1.32E-03			
177-H9	83	HGSHDN	235	.....THPVPA	1.58E-03			
177-H10	83	HGSHDN	236	.....THPTMY	3.44E-03			
144-F1	83	HGSHDN	237	.....HHYTFE	5.80E-04			
43-E3	83	HGSHDN	238	.....SHPAAE	8.00E-04			
43-E9	83	HGSHDN	239	.....TIPSIE	8.00E-04			
43-G2	83	HGSHDN	240	.....SSPAIM	7.00E-04			
43-G3	83	HGSHDN	241	.....IWPNLN	9.00E-04			
31-A6	83	HGSHDN	242	.....THPNLN	5.00E-04			
31-B5	83	HGSHDN	243	.....THPSIS	5.00E-04			
			124	QBYDRGEGTSRY				
Y17	83	HGSHDN	244	QBYDRGSAPIN	8.90E-05	4.50E-10	>1E-8	
Y19	83	HGSHDN	245	QBYDRGHPAMS	2.26E-04	3.00E-11	>1E-8	
Y38	83	HGSHDN	246	.....THPSIT	5.08E-04	5.50E-11	2.60E-09	
Y45	83	HGSHDN	247	.....TOPAIV	6.17E-04	4.00E-11	4.30E-09	
Y61	83	HGSHDN	248	.....THPALL	2.75 e-4	4E-11	1.40E-10	
Y61 IgG	83	HGSHDN	248	.....THPALL	1.50E-04	1.60E-11	1.30E-10	
Y61 IgG germline	83	HGSHDN	248	.....THPALL	1.50E-04	1.60E-11	1.30E-10	1.60E-10
Y139	83	HGSHDN	249	.....SHPALT	5.92E-04	3E-11	4.50E-10	
Y139 IgG1	83	HGSHDN	249	.....SHPALT			1.00E-09	
Y174	83	HGSHDN	250	.....TTPAPE	7.55E-04	6E-11	2.00E-09	
Y177	83	HGSHDN	251	.....SHPALI	6.61E-04	5E-11	1.00E-09	
A5	83	HGSHDN	252	.....THPSML	4.50E-04	6.60E-11		
A12	83	HGSHDN	253	.....TTPRPM	5.57E-04	2.50E-10		
D9	83	HGSHDN	254	.....RLPAQT	8.21E-04	3.5E-09	>>	
G6	83	HGSHDN	255	.....THPLTI	5.08E-04	1E-10	1.00E-09	
G6 IgG1	83	HGSHDN	255	.....THPLTI			1.00E-09	
C6	83	HGSHDN	256	QBYDRGQTPSI	1.07E-03	3.5E-10	1.00E-08	
Y55	83	HGSHDN	257	QBYDRGTHFQMY	1.06E-03	1.40E-10	>1E-8	

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
A4	83	HGSHDN	258	QSYDRGRNPALT	6.30E-04	2.50E-10		
A03	83	HGSHDN	259	QSYDRGTHPLTM	3.04E-04	3.00E-11	4.00E-10	
A03 IgG1	83	HGSHDN	260	QSYDRGTHPLTM	3.04 e-4	2.90E-11	3.80E-10	
A03 IgG germline	83	HGSHDN	260	QSYDRGTHPLTM	2.50E-04	3.50E-11	1.75E-10	
99-B11	83	HGSHDN	261	QSYDSGYTGSRV	5.40E-03			
99-C11	83	HGSHDN	262	QSYDSGFTGSRV	5.70E-03			
99-H4	83	HGSHDN	263	QSYDSRFTGSRV	4.80E-03			
99-E9	83	HGSHDN	262	QSYDSGFTGSRV	5.40E-03			
99-H7	83	HGSHDN	264	QSYDPGTASRV	3.30E-03			
99-H11	83	HGSHDN	265	QSYSTHMPISRV	4.90E-03			
99-F6	83	HGSHDN	266	QSYDSGSTGSRV	4.90E-03			
99-F7	83	HGSHDN	267	QSYNSYPIISRV	4.80E-03			
99-F8	83	HGSHDN	268	QSYRAPQOV	3.70E-03			
99-F11	83	HGSHDN	262	QSYDSGFTGSRV	5.40E-03			
99-G7	83	HGSHDN	269	QSYLKSAFASRV	4.80E-03			
99-G11	83	HGSHDN	270	QSYDSRFTGSRV	4.30E-03			
L3.3R3M-B1	83	HGSHDN	124	QSYDRGFTGSRV				
L3.3R3M-B3	83	HGSHDN	271	.....FTGSMV	5.46E+00			
L3.3R3M-C6	83	HGSHDN	272	.....FTGSMV	5.51E+00			
L3.3R3M-F9	83	HGSHDN	273	.....FTGFDG	6.17E+00			
L3.3R3M-G8	83	HGSHDN	274	.....TAPALS	4.99E+00			
L3.3R3M-H6	83	HGSHDN	275	.....SYPALR	5.55E+00			
L3.3R3M-H10	83	HGSHDN	276	.....NWPNSN	5.69E+00			
L3.3R3M-A3	83	HGSHDN	277	.....TAPSLI	5.35E+00			
L3.3R3M-F8	83	HGSHDN	278	.....FTGSMV	5.37E+00			
L3.3R3M-G1	83	HGSHDN	279	.....TTPRIR	4.99E+00			
L3.3R3M-G7	83	HGSHDN	280	.....FTGSMV	4.21E+00			
L3.3R3M-H11	83	HGSHDN	281	.....FTGSMV	4.24E+00			
	83	HGSHDN	282	.....MIPALT	3.95E+00			

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Table 2

Clone	H3 SEQ ID NO:	H3	L3 SEQ ID NO:	L3	koff	RB assay IC50 (M)	PHA assay IC50 (M)	IFN gamma IC50 (M)
Y61-L94N	109	CKT HGSHDN	283	QSYDRNTHPALL			8.00E-11	
Y61-L94F	109	CKT HGSHDN	284	QSYDRFTHPALL			6.00E-11	
Y61-L94Y	109	CKT HGSHDN	285	QSYDRYTHPALL		2.00E-11	2.00E-11	
Y61-L94Y IgG	109	CKT HGSHDN	285	QSYDRYTHPALL	1.27E-04	6.00E-11	5.00E-11	4.00E-11
Y61-L50Y	109	CKT HGSHDN	286	QSYDRGTHPALL		2.00E-11		2.00E-11
Y61-L50Y* IgG	109	CKT HGSHDN	286	QSYDRGTHPALL	6.98E-05		2.00E-11	3.00E-11
Y61-L50Y-H31E** IgG	109	CKT HGSHDN	286	QSYDRGTHPALL	2.99E-05		6.00E-11	2.00E-11
Y61-L50Y-H31E- L94Y** IgG	109	CKT HGSHDN	287	QSYDRYTHPALL	4.64E-05		1.00E-11	1.00E-11
J695 (Y61-L94Y- L50Y IgG*)	109	CKT HGSHDN	287	QSYDRYTHPALL	5.14E-05	5.00E-11	1.00E-11	5.00E-12

\*CDR L2: L50G to Y

\*\*CDR L2: L50G to Y; CDR H1: H31S to E

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Table 3

Kabat Number	CDR H1					CDR H2					CDR H3				
	27	28	29	30	31	32	33	34	35	50	51	52	52A	53	65
Y61 VH	F	T	F	S	S	Y	G	M	H	F	I	R	Y	D	G
Contact Positions	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hypermutation Positions	x	x	x							x					
Kabat number	CDR L1					CDR L2					CDR L3				
	24	25	26	27A	27B	28	29	30	31	32	33	34	50	51	52
Y61 VL	S	G	G	R	S	N	I	G	S	N	T	V	K	G	N
Contact Positions									x	x	x	x	x	x	x
Hypermutation Positions									x	x	x				
Kabat number	CDR L1					CDR L2					CDR L3				
	89	90	91	92	93	94	95A	95B	95C	96	97	98	99	101	102
Y61 VH	Q	S	Y	D	R	G	T	H	P	A	L	L	L	L	L
Contact Positions	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
Hypermutation Positions															

x contact and/or hypermutation position  
x contact and/or hypermutation position mutated in Y61

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Table 4. Neutralization Activity in the Presence of Excess Free IL-12 p40

SEQ ID NO:	Clone	PHA assay IC50 (M) p70:p40 1:0	PHA assay IC50 (M) p70:p40 1:20	PHA assay IC50 (M) p70:p40 1:50
VH: 47	136-15	2.00E-09	5.00E-09	4.00E-09
VL: 48				
VH: 51	149-5	6.50E-09	7.00E-09	4.00E-09
VL: 52				
VH: 53	149-6	9.00E-10	1.00E-09	1.00E-09
VL: 54				
VH: 84	149-7	3.50E-09	2.50E-09	4.00E-09
VL: 126				
VH: 23	Y61 IgG	1.80E-10		1.80E-10
VL: 24				
VH: 65	AO3 IgG1	2.50E-10		2.20E-10
VL: 66				
VH: 31	J695	1.00E-11		3.50E-11
VL: 32				

## EXAMPLES

### **EXAMPLE 1: Isolation of Anti-IL-12 Antibodies**

#### 5 A. Screening for IL-12 binding antibodies

Antibodies to hIL-12 were isolated by screening three separate scFv phage display libraries prepared using human VL and VH cDNAs from mRNA derived from human tonsils (referred to as scFv 1), tonsil and peripheral blood lymphocytes (PBL) (referred to as scFv 2), and bone marrow-derived lymphocytes (referred to as  
10 BMDL). Construction of the library and methods for selection are described in Vaughan et al. (1996) Nature Biotech. 14: 309-314.

The libraries were screened using the antigens, human IL-12 p70 subunit, human IL-12 p40 subunit, chimaeric IL-12 (mouse p40/human p35), mouse IL-12, biotinylated human IL-12 and biotinylated chimaeric IL-12. IL-12 specific antibodies were selected  
15 by coating the antigen onto immunotubes using standard procedures (Marks et al., (1991) J. Mol. Biol. 222: 581-597). The scFv library 2 was screened using either IL-12, or biotinylated-IL-12, and generated a significant number of IL-12 specific binders. Five different clonotypes were selected, determined by BstNI enzymatic digestion patterns, and confirmed by DNA sequencing. The main clonotypes were  
20 VHDP58/VLDPL11, VHDP77/VLDPK31, VHDP47/VL and VHDP77/VLDPK31, all of which recognized the p40 subunit of IL-12.

Screening of the BMDL library with IL-12 p70 generated 3 different clonotypes. Two of these were found to be cross-reactive clones. The dominant clone was sequenced and consisted of VHDP35/VLDP. This clone recognizes the p40 subunit of  
25 IL-12. Screening of the scFv library 1, using IL-12 p70, did not produce specific IL-12 antibodies.

In order to identify IL-12 antibodies which preferentially bind to the p70 heterodimer or the p35 subunit of IL-12, rather than the p40 subunit, the combined scFv 1 + 2 library, and the BMDL library were used. To select IL-12 antibodies that  
30 recognized the p70 heterodimer or p35 subunit, phage libraries were preincubated and selected in the presence of free p40. Sequencing of isolated clones revealed 9 different antibody lineages. Subunit preferences were further analyzed by 'micro-Friguet'

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titration. The supernatant containing scFv was titrated on biotin-captured IL-12 in an ELISA and the ED<sub>50</sub> determined. The concentration of scFv producing 50% ED was preincubated with increasing concentrations of free p70 or p40 (inhibitors). A decrease in the ELISA signal on biotin-IL-12 coated plates was measured and plotted against the concentration of free p70 or p40. This provided the IC<sub>50</sub> for each clone with respect to p70 and p40. If the titrations for both subunits overlaps, then the scFv binds to both p40 and p70. Any variation from this gives the degree of preference of p70 over p40.

#### B. Affinity Maturation of Antibody Lineage Specific for IL-12 (Joe 9)

The clones were tested for their ability to inhibit IL-12 binding to its receptor in an IL-12 receptor binding assay (referred to as RBA), and for their ability to inhibit IL-12 induced proliferation of PHA stimulated human blast cells (PHA assay), described in Example 3. Clone Joe 9 had the lowest IC<sub>50</sub> value in both the RBA and the PHA assay, with an IC<sub>50</sub> value of  $1 \times 10^{-6}$  M in both assays. In addition the heavy chain variable region (VH) of Joe 9 had the least number of changes compared to the closest germline sequence COS-3, identified from the VBASE database. Table 1 (see Appendix A) shows the V<sub>H</sub>3 family of germline sequences, of which COS-3 is a member, as well as members of V<sub>L</sub>1 family of germline sequences. Therefore, Joe 9 was selected for affinity maturation. The amino acids sequences of VH and VL of the Joe9 wild type (Joe9 wt) antibody are shown in Figure 1A-1D.

In order to increase the affinity of Joe 9, various mutations of the complementarity determining region 3 (CDR3) of both the heavy and light chains were made. The CDR3 variants were created by site-directed PCR mutagenesis using degenerate oligonucleotides specific for either the heavy chain CDR3 (referred to as "H3") or the light chain CDR3 (referred to as "L3"), with an average of three base substitutions in each CDR3 (referred to as "spike"). PCR mutagenesis of the heavy chain CDR3 was performed using the degenerate heavy chain oligonucleotide containing a random mixture of all four nucleotides,

5'TGTCCCTTGGCCCCA(G)(T)(A)(G)(T)(C)(A)(T)(A)(G)(C)(T)(C)(C)(A)(C)(T)  
GGTCGTACAGTAATA 3' (SEQ ID NO: 580), and oligonucleotide pUC Reverse Tag  
GAC ACC TCG ATC AGC GGA TAA CAA TTTCAC ACA GG (SEQ ID NO: 581)



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to generate a repertoire of heavy chain CDR3 mutants. The parent light chain was amplified using Joe 9 reverse oligonucleotide (5'TGG GGC CAA GGG ACA3' (SEQ ID NO:582) and the fdteteseq 24+21 oligonucleotide (5'-ATT CGT CCT ATA CCG TTC TAC TTT GTC GTC TTT CCA GAC GTT AGT-3' (SEQ ID NO: 583).

5 Complementarity between the two PCR products was used to drive annealing of the two fragments in a PCR assembly reaction and the full length recombined scFv library was amplified with pUC Reverse Tag (SEQ ID NO: 581) and fdTag 5'-ATT CGT CCT ATA CCG TTC-3' (SEQ ID NO: 584). PCR mutagenesis of the light chain, was performed using the light chain oligonucleotide containing a mixture of all four  
10 nucleotides  
5'GGTCCCAGTTCCGAAGACCCTCGAACC(C)(C)(T)(C)(A)(G)(G)(C)(T)  
(G)(C)(T)(G)(T)(C)ATATGACTGGCAGTAATAGTCAGC 3' (SEQ ID NO: 585), and Joe 9 reverse oligonucleotide 5'TGG GGC CAA GGG ACA3' (SEQ ID NO: 586) to produce a repertoire of light chain CDR3 mutants. The parent heavy chain was  
15 amplified with pUC Reverse Tag (SEQ ID NO: 581) and HuJH3FOR oligonucleotide 5'TGAAGAGACGGTGACCATTGTCCC3' (SEQ ID NO: 587). Complementarity between the two PCR products was used to drive annealing of the two fragments in a PCR assembly reaction and the full length recombined scFv library was amplified with Reverse Tag GAC ACC TCG ATC AGC G (SEQ ID NO: 588) and HuJλ 2-3 FOR  
20 NOT oligonucleotide 5'GAG TCA TTC TCG ACT TGC GGC CGC ACC TAG GAC GGT CAG CTT GGT CCC 3' (SEQ ID NO: 589).

Heavy chain CDR3 mutants were selected using 1 nM biotinylated IL-12, and washed for 1 h at room temperature in PBS containing free IL-12 or p40 at a concentration of 7 nM. Clones were analyzed by phage ELISA and those that bound to  
25 IL-12 were tested in BIAcore kinetic binding studies using a low density IL-12 chip (see procedure for BIAcore analysis in Example 5). Generally, BIAcore analysis measures real-time binding interactions between ligand (recombinant human IL-12 immobilized on a biosensor matrix) and analyte (antibodies in solution) by surface plasmon resonance (SPR) using the BIAcore system (Pharmacia Biosensor, Piscataway, NJ). The system  
30 utilizes the optical properties of SPR to detect alterations in protein concentrations within a dextran biosensor matrix. Proteins are covalently bound to the dextran matrix at known concentrations. Antibodies are injected through the dextran matrix and

specific binding between injected antibodies and immobilized ligand results in an increased matrix protein concentration and resultant change in the SPR signal. These changes in SPR signal are recorded as resonance units (RU) and are displayed with respect to time along the y-axis of a sensorgram. To determine the off rate ( $k_{off}$ ), on rate ( $k_{on}$ ), association rate ( $K_a$ ) and dissociation rate ( $K_d$ ) constants, BIAcore kinetic evaluation software (version 2.1) was used. Clones that demonstrated an improvement in the  $k_{off}$  rate were analyzed by neutralization assays which included inhibition by antibody of IL-12 binding to its receptor (RBA assay), inhibition of IL-12-induced proliferation in PHA stimulated human blast cells (PHA assay), and inhibition of IL-12-induced interferon gamma production by human blast cells (IFN gamma assay). A summary of the dissociation rates and/or  $IC_{50}$  values from neutralization assays of heavy chain CDR3 spiked clones 70-1 through 70-13 is presented in Table 2 (see Appendix A). Clone 70-1 displayed a  $k_{off}$  rate that was better than the parent Joe 9 clone, and had the lowest  $IC_{50}$  value of  $2.0 \times 10^{-7}$  M. Therefore clone 70-1 was selected for conversion to complete IgG1.

Light chain CDR3 mutants were selected using 1 nM biotin-IL-12 and washed with PBS containing 7 nM free p40. Clones were screened in phage ELISA and those that bound to IL-12 were tested in BIAcore binding analysis using low density IL-12 chips. Clones that displayed an off rate which was better than the parent Joe 9 clone were tested in neutralization assays which measured either, inhibition of IL-12 receptor binding, or inhibition of PHA blast cell proliferation. A summary of the dissociation rates and/or  $IC_{50}$  values from neutralization assays of light chain CDR3 mutant clones, 78-34 through 79-1, is presented in Table 2 (see Appendix A).

Based on the  $k_{off}$  rate, clones 78-34 and 78-35 displayed an improved  $k_{off}$  rate compared to the parent Joe 9. Both of these clones were selected for combination analysis with heavy chain mutants.

### C. Combination Clones

Mutant light and heavy chain clones that exhibited the best binding characteristics were used for combination and assembly of scFvs. Mutant clones with improved potency characteristics were combined by PCR overlap extension and pull-

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through of the mutated VH and VL segments as described above. Clones 101-14 through 26-1, shown in Table 2 (see Appendix A), were produced from the combination of heavy chain mutants (70-2, 70-13 and 70-1) with light chain mutants (78-34, 78-35 and 79-1). The  $k_{off}$  rates and/or  $IC_{50}$  values from neutralization assays for these clones are presented in Table 2.

BIAcore binding analysis identified clone 101-11, produced from the combination of the heavy chain CDR3 mutant clone 70-1 with the light chain CDR3 mutant clone 78-34, as having an off rate of  $0.0045 s^{-1}$ . This  $k_{off}$  rate was a significant improvement compared to the  $k_{off}$  rates for either the heavy chain CDR3 mutant clone 70-1 ( $0.0134 s^{-1}$ ), or for the light chain CDR3 mutant clone 78-34 ( $0.0164 s^{-1}$ ) alone. Furthermore, clone 101-11 showed a significant improvement in neutralization assays. Accordingly, clone 101-11 was selected for affinity maturation as described below.

D. Affinity maturation of clone 101-11

Further affinity maturation of clone 101-11 consisted of repeat cycles of PCR mutagenesis of both the heavy and light chain CDR3s of 101-11 using spiked oligonucleotide primers. The clones were selected with decreasing concentrations of biotinylated IL-12 (bio-IL-12). The binding characteristics of the mutated clones was assessed by BIAcore binding analysis and RBA, PHA neutralization assays. The  $k_{off}$  rates and/or  $IC_{50}$  values for clones 136-9 through 170-25 are presented in Table 2 (see Appendix A). Clone 103-14 demonstrated an improved  $IC_{50}$  value in both the receptor binding assay and the PHA blast assay. Clone 103-14 also demonstrated a low  $k_{off}$  rate, and accordingly was selected for further affinity maturation.

E. Generation and Selection of Randomized Libraries of Clone 103-14 Light CDR3

The light chain CDR3 of clone 103-14 (QSYDRGFTGSMV (SEQ ID NO: 590)) was systematically randomized in 3 segments using 3 different libraries as outlined below, where X is encoded by a randomized codon of sequence NNS with N being any nucleotide and S being either deoxycytosine or deoxyguanine.

L3.1=XXXXXXFTGSMV (SEQ ID NO: 591)

L3.2= QSYXXXXXXSMV (SEQ ID NO: 592)

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L3.3=QSYDRGXXXXXX (SEQ ID NO: 593)

Randomized mutagenesis of all three light chain CDRs (referred to as L3.1, L3.2, and L3.3) of clone 103-14 was performed. The heavy chain CDR3 (referred to as H3) of clone 103-14 was not mutated. Four randomized libraries based on clone 103-14  
5 (H3 and L3.1, L3.2 & L3.3) were constructed and subjected to a large variety of selection conditions that involved using limiting antigen concentration and the presence or absence of excess free antigen (p40 and p70). The outputs from selections (clones 73-B1 through 99-G11) were screened primarily by BIAcore, and on occasion with RBA and are shown in Table 2 (see Appendix A).

10 Random mutagenesis of the light chain CDR of 103-14 generated clone Y61, which exhibited a significant improvement in IC<sub>50</sub> value compared to the parent clone 103-14. Y61 was selected for conversion to a whole IgG1. Whole Y61-IgG1 has an IC<sub>50</sub> value of approximately 130 pM determined by the PHA assay. The IC<sub>50</sub> value was not affected by a 50 fold molar excess of free p40, demonstrating that free p40 did not  
15 cross-react with Y61 anti-IL-12 antibody to thereby decrease the antibody binding to the heterodimer. The full length sequences of Y61 heavy chain variable region and light chain variable region are shown below.

20 Y61 Heavy Chain Variable Region Peptide Sequence

**CDR H1**

QVQLVESGGGVVQPGRSLRLSCAASFTFS SYGMH WVRQAPGKGLEWVA

25 **CDR H2**

FIRYDGSNKYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCKT

**CDR H3**

HGSHDN WGQGTMTVTVSS (SEQ ID NO: 23)

30

Y61 Light Chain Variable Region Peptide Sequence

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**CDR L1**

QSVLTQPPSVSGAPGQRTISC SGGRSNIGSNTVK WYQQLPGTAPKLLIY

**CDR L2**

5 GNDQRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC

**CDR L3**

QSYDRGTHPALL FGTGTKVTVLG (SEQ ID NO:24)

10 CDR residues are assigned according to the Kabat definitions.

**EXAMPLE 2: Mutation of Y61 at Hypermutation and Contact Positions**

Typically selection of recombinant antibodies with improved affinities can be carried out using phage display methods. This is accomplished by randomly mutating combinations of CDR residues to generate large libraries containing single-chain antibodies of different sequences. Typically, antibodies with improved affinities are selected based on their ability to reach an equilibrium in an antibody-antigen reaction. However, when Y61 scFV was expressed on phage surface and incubated with IL-12, selection conditions could not be found that would allow the system to reach normal antibody-antigen equilibrium. The scFV-phage remained bound to IL-12, presumably due to a non-specific interaction, since purified Y61 scFv exhibits normal dissociation kinetics. Since the usual methods of phage-display affinity maturation to Y61 (i.e. library generation and selections by mutagenesis of multiple CDR residues) could not be utilized, a new strategy was developed in which individual CDR positions were mutated.

This strategy involves selection of appropriate CDR positions for mutation and is based on identification and selection of amino acids that are preferred selective mutagenesis positions, contact positions, and/or hypermutation positions. Contact positions are defined as residues that have a high probability of contact with an antigen when the antigen interacts with the antibody, while hypermutation positions are defined as residues considered to have a high probability for somatic hypermutation during *in vivo* affinity maturation of the antibody. Preferred selective mutagenesis positions are CDR positions that are both contact and hypermutation positions. The Y61 antibody

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was already optimized in the CDR3 regions using the procedure described in Example 1, therefore it was difficult to further improve the area which lies at the center of the antibody binding site using phage-display selection methods. Greater improvements in activity were obtained by mutation of potential contact positions outside the CDR3 regions by either removing a detrimental antigen-antibody contact or, engineering a new contact.

Amino acids residues of Y61 which were considered contact points with antigen, and those CDR positions which are sites of somatic hypermutations during *in vivo* affinity maturation, are shown in Table 3 (see Appendix A). For Y61 affinity maturation, 15 residues outside CDR3, 3 residues within the L3 loop, and 5 residues in the H3 loop were selected for PCR mutagenesis.

Y61 scFv gene was cloned into the pUC119(Sfi) plasmid vector for mutagenesis. Oligonucleotides were designed and synthesized with randomized codons to mutate each selected position. Following PCR mutagenesis, a small number of clones (~24) were sequenced and expressed in a host cell, for example, in a bacterial, yeast or mammalian host cell. The expressed antibody was purified and the  $k_{off}$  measured using the BIAcore system. Clones with improved off-rates, as compared to Y61, were then tested in neutralization assays. This procedure was repeated for other CDR positions. Individual mutations shown to have improved neutralization activity were combined to generate an antibody with even greater neutralization potency.

The Y61 CDR positions that were mutated in order to improve neutralization potency, and the respective amino-acid substitutions at each position are shown in Figures 2A-2H. Off-rates, as determined by BIAcore analysis, are given. These off rates are also shown in the histograms to the right of each table.

Results of these substitutions at positions H30, H32, H33, H50, H53, H54, H58, H95, H97, H101, L50, L92, L93, demonstrated that all amino-acid substitutions examined resulted in antibodies with poorer off-rates than Y61. At positions H52, L32, and L50, only a one amino acid substitution was found to improve the off-rate of Y61, all other changes adversely affected activity. For L50, this single Gly→Tyr change significantly (5-10 times) improved the neutralization potency of Y61. The results demonstrated the importance of these positions to Y61 activity, and suggest that in most cases phage-display was able to select for the optimal residues. However, at positions

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H31, H56, L30, and L94, several substitutions were found to improve Y61 off-rate, suggesting that these positions were also important for antigen binding, although the phage display approach did not allow selection of the optimal residues.

Selective mutation of contact and hypermutation positions of Y61 identified amino acid residue L50 in the light chain CDR2, and residue L94 of the light chain CDR3, which improved the neutralization ability of Y61. A combination of these mutations produced an additive effect, generating an antibody, J695, that exhibited a significant increase in neutralization ability. The full length sequence of J695 heavy and light chain variable region sequences is shown below.

10

J695 Heavy Chain Variable Region Peptide Sequence

**CDR H1**

QVQLVESGGGVVQPGRSLRLSCAASGFTFS SYGMH WVRQAPGKGLEWVA

15

**CDR H2**

FIRYDGSNKYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCKT

**CDR H3**

20 HGSHDN WGQGTMTVTSS (SEQ ID NO: 31)

J695 Light Chain Variable Region Peptide Sequence

25

**CDR L1**

QSVLTQPPSVSGAPGQRVTISC SGSRSNIGSNTVK WYQQLPGTAPKLLIY

**CDR L2**

YNDQRPS GVPDRFSGSKSGTSASLAITGLQAEDEADYYC

30

**CDR L3**

QSYDRYTHPALL FGTGTVTVLG (SEQ ID NO: 32)

CDR residues are assigned according to the Kabat definitions.

A summary of the heavy and light chain variable region sequence alignments showing the lineage development of clones that were on the path from Joe9 to J695 is shown in Figures 1A-1D. The CDRs and residue numbering are according to Kabat.

### EXAMPLE 3: Functional Activity of Anti-hIL-12 Antibodies

To examine the functional activity of the human anti-human IL-12 antibodies of the invention, the antibodies were used in several assays that measure the ability of an antibody to inhibit IL-12 activity.

#### A. Preparation of Human PHA-activated Lymphoblasts

Human peripheral blood mononuclear cells (PBMC) were isolated from a leukopac collected from a healthy donor by Ficoll-Hypaque gradient centrifugation for 45 minutes at 1500 rpm as described in Current Protocols in Immunology, Unit 7.1. PBMC at the interface of the aqueous blood solution and the lymphocyte separation medium were collected and washed three times with phosphate-buffered saline (PBS) by centrifugation for 15 minutes at 1500 rpm to remove Ficoll-Paque particles.

The PBMC were then activated to form lymphoblasts as described in Current Protocols in Immunology, Unit 6.16. The washed PBMC were resuspended at  $0.5 \times 10^6$  cells/ml in RPMI complete medium (RPMI 1640 medium, 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin), supplemented with 0.2% (v/v) PHA-P (Difco, Detroit, MI) and cultured for four days at 37°C in a 5% CO<sub>2</sub> atmosphere. After four days, cell cultures were split 1:1 by volume in RPMI complete medium, plus 0.2% (v/v) PHA-P and 50 U/ml recombinant human IL-2. Recombinant human IL-2 was produced by transfection of an expression vector carrying the human IL-2 cDNA into COS cells (see Kaufman *et al.*, (1991) *Nucleic Acids Res.* 19, 4484-4490), and purified as described in PCT/US96/01382. Cell cultures were then incubated for an additional one to three days. PHA blast cells were harvested, washed twice with RPMI complete medium and frozen in 95% FBS, 5% DMSO at  $10 \times 10^6$  cells/ml.



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PHA blast cells to be used for the IL-12 receptor binding assay (see section B) were collected after one day culture in the presence of IL-2, whereas PHA blast cells to be used for the PHA blast proliferation assay (see section C) and the interferon-gamma induction assay (see section D) were collected after three day culture in the presence of IL-2.

#### B. IL-12 Receptor Binding Assay

The ability of anti-IL-12 antibodies to inhibit binding of radiolabelled IL-12 to IL-12 receptors on PHA blasts were analyzed as follows. Various concentrations of anti-IL-12 antibody were preincubated for 1 hour at 37°C with 50-100 pM  $^{125}$ I-hIL-12 (iodinated hIL-12 was prepared using the Bolton-Hunter labeling method to a specific activity of 20-40mCi/mg from NEN-Dupont) in binding buffer (RPMI 1640, 5% FBS, 25 mM Hepes pH 7.4). PHA blast cells isolated as described above, were washed once and resuspended in binding buffer to a cell density of  $2 \times 10^7$  cells/ml. PHA blasts ( $1 \times 10^6$  cells) were added to the antibody  $^{125}$ I-hIL-12 mixture and incubated for two hours at room temperature. Cell bound radioactivity was separated from free  $^{125}$ I-hIL-12 by centrifugation of the assay mixture for 30 seconds at room temperature, aspiration of the liquid and a wash with 0.1ml binding buffer, followed by centrifugation at 4°C for 4 min at 10,000 x g. The cell pellet was examined for cell bound radioactivity using a gamma counter. Total binding was determined in the absence of antibody and non-specific binding was determined by inclusion of 25 nM unlabeled IL-12 in the assay. Incubations were carried out in duplicate.

In the IL-12 receptor binding assay using the Y61 and J695 human anti-IL-12 antibodies, both antibodies demonstrated a comparable inhibition of IL-12 receptor binding. Y61 inhibited IL-12 receptor binding with an  $IC_{50}$  value of approximately  $1.6 \times 10^{-11}$ M, while J695 had an  $IC_{50}$  value of approximately  $1.1 \times 10^{-11}$ M.

### C. Human PHA Blast Proliferation Assay

Anti-IL-12 antibodies were evaluated for their ability to inhibit PHA blast proliferation (which proliferation is stimulated by IL-12). Serial dilutions of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO<sub>2</sub> with 230 pg/ml hIL-12 in  
5 100 ml RPMI complete medium in a microtiter plate (U-bottom, 96-well, Costar, Cambridge, MA). PHA blast cells isolated as described above, were washed once and resuspended in RPMI complete medium to a cell density of  $3 \times 10^5$  cells/ml. PHA blasts (100 ml,  $3 \times 10^4$  cells) were added to the antibody/hIL-12 mixture, incubated for 3 days at 37°C, 5% CO<sub>2</sub> and labeled for 4-6 hours with 0.5 mCi/well (3H)-Thymidine  
10 (Amersham, Arlington Heights, IL). The culture contents were harvested onto glass fiber filters by means of a cell harvester (Tomtec, Orange, CT) and (<sup>3</sup>H)-Thymidine incorporation into cellular DNA was measured by liquid scintillation counting. All samples were assayed in duplicate.

The results of neutralization in the presence of varying concentrations of p70:p40  
15 (i.e. the ratio of IL-12 heterodimer to free p40 subunit) is shown in Table 4 (see Appendix A).

Analysis of the Y61 human anti-IL-12 antibody in the PHA blast proliferation assay demonstrated that the antibody inhibited PHA blast proliferation with an IC<sub>50</sub> value of approximately  $1.8 \times 10^{-10}$  M in the presence of IL-12 p70 alone, without any  
20 excess p40 (p70:p40 ratio of 1:0). In the presence of a 50-fold excess of free p40 (p70:p40 at a ratio of 1:50), the Y61 antibody inhibited PHA blast proliferation with an IC<sub>50</sub> value of approximately  $1.8 \times 10^{-10}$  M. This result demonstrates that the ability of Y61 to inhibit blast proliferation is not compromised by the presence of excess p40.

The human anti-IL-12 antibody, J695 inhibited PHA blast proliferation with an  
25 IC<sub>50</sub> value of approximately  $1.0 \times 10^{-11}$  M in the presence of p70:p40 at a ratio of 1:0. In the presence of a p70:p40 ratio of 1:50, this antibody inhibited PHA blast proliferation with an IC<sub>50</sub> value of approximately  $5.8 \pm 2.8 \times 10^{-12}$  M (n=2), demonstrating that the excess p40 had only a slight inhibitory effect on the antibody. Overall results demonstrate the improved neutralization activity of J695 in comparison with Y61 due to  
30 the mutations at L50 and L94.

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D. Interferon-gamma Induction Assay

The ability of anti-IL-12 antibodies to inhibit the production of IFN $\gamma$  by PHA blasts (which production is stimulated by IL-12) was analyzed as follows. Various concentrations of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO<sub>2</sub> with 200-400 pg/ml hIL-12 in 100  $\mu$ l RPMI complete medium in a microtiter plate (U-bottom, 96-well, Costar). PHA blast cells isolated as described above, were washed once and resuspended in RPMI complete medium to a cell density of  $1 \times 10^7$  cells/ml. PHA blasts (100  $\mu$ l of  $1 \times 10^6$  cells) were added to the antibody/hIL-12 mixture and incubated for 18 hours at 37°C and 5% CO<sub>2</sub>. After incubation, 150  $\mu$ l of cell free supernatant was withdrawn from each well and the level of human IFN $\gamma$  produced was measured by ELISA (Endogen Interferon gamma ELISA, Endogen, Cambridge, MA). Each supernatant was assayed in duplicate.

Analysis of human anti-hIL-12 antibody, Y61 in this assay demonstrated that Y61 inhibited human IFN $\gamma$  production with an IC<sub>50</sub> value of approximately  $1.6 \times 10^{-10}$  M, while the human anti-IL-12 antibody, J695, inhibited human IFN $\gamma$  production with an IC<sub>50</sub> value of approximately  $5.0 \pm 2.3 \times 10^{-12}$  M (n=3). The result demonstrates the substantial improvement in the affinity of J695 as a result of the modifications at L50 and L94.

E. Induction of Non-human IL-12 from Isolated PBMC

To examine the cross-reactivity of the human anti-hIL-12 antibodies with IL-12 from other species, non-human IL-12 was produced as follows. PBMC were separated from fresh heparinized blood by density gradient centrifugation as described above using lymphoprep (Nycomed, Oslo, Norway) for cynomolgus monkey, baboon, and dog, PBMC, Accu-paque (Accurate Chemical & Sci. Corp., Westbury, NY) for dog PBMC or Lympholyte-rat (Accurate Chemical & Sci. Corp., Westbury, NY) for rat PBMC.

The PBMC were then induced to produce IL-12 as described (D'Andrea *et al.*, (1992) *J. Exp. Med.* 176, 1387-1398, Villinger *et al.*, (1995) *J. Immunol.* 155, 3946-3954, Buettner *et al.*, (1998) *Cytokine* 10, 241-248). The washed PBMC were resuspended at  $1 \times 10^6$  cells/ml in RPMI complete medium, supplemented with 0.0075% (wt/vol) of SAC (Pansorbin; Calbiochem-Behring Co., La Jolla, CA) or 1-5 mg/ml

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ConA (Sigma Chemical Co., St. Louis, MO) plus 0.0075% SAC and incubated for 18 hours at 37°C in a 5% CO<sub>2</sub> atmosphere. Cell-free and SAC-free medium was collected by centrifugation and filtering through 0.2 mm filters.

IL-12 from the rhesus monkey was obtained as recombinant rhesus IL-12 from  
5 Emory University School of Medicine, Atlanta, GA.

F. Murine 2D6 Cell Proliferation Assay

The murine T cell clone 2D6 proliferates in response to murine IL-2, IL-4, IL-7 and IL-12 (Maruo *et al.*, (1997) *J. Leukocyte Biol.* 61, 346-352). A significant  
10 proliferation was also detected in response to rat PBMC supernatants containing rat IL-12. The cells do not respond to dog, cynomolgus, baboon or human IL-12. Murine 2D6 cells were propagated in RPMI complete medium supplemented with 50 mM beta-mercaptoethanol ( $\beta$ ME) and 30 ng/ml murine IL-12. One day prior to the assay, the murine IL-12 was washed out and the cells were incubated overnight in RPMI complete  
15 medium plus  $\beta$ ME.

Serial dilutions of anti-IL-12 antibody were preincubated for 1 hour at 37°C, 5% CO<sub>2</sub> with 40 pg/ml murine IL-12 in 100 ml RPMI complete medium plus  $\beta$ ME in a microtiter plate (U-bottom, 96-well, Costar). 2D6 cells were washed once and resuspended in RPMI complete medium containing  $\beta$ ME to a cell density of  $1 \times 10^5$   
20 cells/ml. 2D6 cells (100  $\mu$ l,  $1 \times 10^4$  cells) were added to the antibody/hIL-12 mixture, incubated for 3 days at 37°C, 5% CO<sub>2</sub> and labeled for 4-6 hours with 0.5 mCi/well (3H)-Thymidine. The culture contents were harvested and counted by liquid scintillation counting. All samples were assayed in duplicate.

25 G. Species Cross-reactivity of J695 with Non-Human IL-12

Species cross-reactivity of J695 with non-human IL-12 was analyzed using PBMC's isolated from several non-human species. The presence of non-human IL-12 activity in the rat, dog, cynomolgus and baboon PBMC supernatants was confirmed using several bioassays described above, such as the murine 2D6 cell proliferation assay,  
30 the human PHA blast proliferation assay and the interferon-gamma induction assay by blocking the non-human PBMC induced responses with rabbit and/or sheep polyclonal

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antibodies to murine and/or human IL-12. Cross-reactivity of the human anti-hIL-12 antibodies Y61 and J695 with non-human IL-12 in PBMC supernatants or purified murine and rhesus IL-12 was then assessed in the same bioassay(s) by determining the J695 antibody concentration at which 50% inhibition of the response was observed. The species cross-reactivity results are summarized in Table 5. The results demonstrate that Y61 and J695 are each able to recognize IL-12 from monkeys (e.g. cynomolgus and rhesus IL-12 for Y61, and cynomolgus, rhesus and baboon for J695) and that J695 is approximately 35 fold less active on dog IL-12; neither Y61 nor J695 cross reacts with mouse or rat IL-12.

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#### H. Human cytokine specificity of J695

The specificity of J695 was tested in a competition ELISA in which a panel of human cytokines was tested for their ability to interfere with the binding of soluble J695 to immobilized human IL-12. The panel of human cytokines included IL-1 $\alpha$  and IL-1 $\beta$  (Genzyme, Boston, MA), IL-2 (Endogen), IL-4, IL-10, IL-17, IFN-gamma, and TGF- $\beta$ 1 (R&D, Minneapolis, MN) IL-8 (Calbiochem), PDGF, IGF-I, and IGF-II (Boehringer Mannheim Corp., Indianapolis, IN), TNF $\alpha$  and lymphotoxin, IL-6, soluble IL-6 receptor, IL-11, IL-12 p70, IL-12 p40, M-CSF, and LIF. EBI-3, an IL-12 p40 related protein that is induced by Epstein-Barr virus infection in B lymphocytes (Devergne *et*

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Table 5 Species Cross Reactivity Data

Antibody		IC <sub>50</sub> (M)						
Name	Specificity	Mouse IL-12	Rat IL-12	Dog IL-12	Cyno IL-12	Rhesus IL-12	Baboon IL-12	Human IL-12
		Purified	PBMC sup	PBMC sup	PBMC sup	Purified	PBMC sup	Purified
C17.15	rat- $\alpha$ mulL12	$3.0 \times 10^{-11}$						
R03B03	rabbit- $\alpha$ mulL12	$1.5 \times 10^{-10}$	$6.0 \times 10^{-10}$					
C8.6.2	mouse- $\alpha$ mulL12				$1.2 \times 10^{-10}$	$1.0 \times 10^{-10}$	$2.0 \times 10^{-10}$	$5.0 \times 10^{-11}$
Y61	human- $\alpha$ mulL12	Non-neutralizing			$2.2 \times 10^{-10}$	$1.0 \times 10^{-10}$		$1.7 \times 10^{-10}$
J695	human- $\alpha$ mulL12	Non-neutralizing	Non-neutralizing	$3.5 \times 10^{-10}$	$1.0 \times 10^{-11}$	$1.0 \times 10^{-11}$	$1.5 \times 10^{-11}$	$5.0 \times 10^{-12}$

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*al.*, (1996) *J. Virol.* 70, 1143-1153 ) was expressed as a human IgG-Fc chimera (EBI-3/Fc) Single-stranded salmon sperm DNA (Sigma) was also tested.

Flat-bottom ELISA immunoassay microtiter plates (96 well, high binding, Costar) were coated overnight at 4°C with 0.1 ml human IL-12 (2 µg/ml in 0.1 M carbonate coating buffer (4 volumes 0.1 M NaHCO<sub>3</sub> plus 8.5 volumes 0.1 M NaHCO<sub>3</sub>)). The plates were washed twice with PBS containing 0.05 % Tween 20 (PBS-T), blocked with 200µl of 1 mg/ml bovine serum albumin (BSA, Sigma) in PBS-T for 1 hour at room temperature, and again washed twice with PBS-T. Samples (100 µl) containing IL-12 antibody J695 (100 ng/ml) and each cytokine (2nM) in PBS-T containing 50 µg/ml BSA (PBS-T/BSA) were added and incubated for 2 h at room temperature. The plates were washed 4 times and incubated for 1 h at room temperature with 100 µl mouse anti-human lambda-HRP (1:500 in PBS-T/BSA, Southern Biotech. Ass. Inc., Birmingham, AL). The plates were washed 4 times and developed with ABTS (Kirkegaard & Perry Lab., Gaithersburg, MD) for 20-30 minutes in the dark. The OD<sub>450nm</sub> was read using a microplate reader (Molecular Devices, Menlo Park, CA). Percent binding was determined relative to J695 binding to the IL-12 coated plate in the absence of any soluble cytokine.

The results demonstrated that J695 binding to immobilized human IL-12 was blocked only by human IL-12 p70 and to a lesser extent, by human IL-12 p40 and not by any of the other cytokines tested.

#### I. Binding to a Novel IL-12 Molecule

An alternative IL-12 heterodimer has been described, in which the p35 subunit is replaced by a novel p19 molecule. P19 was identified using 3D homology searching for IL-6/IL-12 family members, and is synthesized by activated dendritic cells. P19 binds to p40 to form a p19/p40 dimer, which has IL-12 -like activity, but is not as potent as the p35/p40 heterodimer in IFNγ induction. Antibodies which recognize p40 alone, but preferably in the context of a p70 molecule (e.g., J695 and Y61, see Example 3H) are expected to also neutralize both the p35/p40 molecules and the p19/p40 molecules.

**EXAMPLE 4: In vivo Activity of Anti-hIL-12 Antibodies**

The *in vivo* effects of IL-12 antibodies on IL-12 induced responses were examined in a model modified from one used by Bree *et al.* to study the effect of human IL-12 on peripheral hematology in cynomolgus monkey Bree *et al.*, (1994) *Biochem Biophys Res. Comm.* 204: 1150-1157. In those previous studies, administration of human IL-12 at 1 µg/kg/day for a period of 5 days resulted in a decrease in white blood cell count (WBC), especially in the lymphocyte and monocyte subsets after 24 hours. A decrease in the platelet count was observed at 72 hours. Levels of plasma neopterin, a marker of monocyte activation in response to IFN-γ, began to elevate at 24 hours and were the highest at 72 hours.

In the first study with human anti-hIL-12 antibodies, fifteen healthy cynomolgus monkeys with an average weight of 5kg, were sedated and divided into 5 groups (n=3). Group 1 received an intravenous (IV) administration of 10 mg/kg human intravenous immunoglobulin (IVIG, Miles, Eckhart, IN, purified using protein A Sepharose). Group 2 received an intravenous administration of 1 mg/kg C8.6.2 (neutralizing mouse anti-human IL-12 monoclonal antibody). Group 3 received an intravenous administration of 10 mg/kg C8.6.2. Group 4 received an intravenous administration of 1 mg/kg Y61 (human anti-human IL-12 antibody, purified from CHO cell conditioned medium). Group 5 received an intravenous administration of 10 mg/kg Y61.

One hour after the antibody administration all animals received a single subcutaneous (SC) injection of human IL-12 (1 µg/kg). Blood samples were taken at the following time points: baseline, 8, 24, 48, 96 and 216 hours, and analyzed for complete blood cell counts with differentials and serum chemistry. Serum human IL-12, C8.6.2 antibody, Y61 antibody, monkey IFN-gamma, monkey IL-10, monkey IL-6 and plasma neopterin levels were also measured.

Animals treated with IL-12 plus IVIG control antibody (Group 1) showed many of the expected hematological changes, including decreases in WBC, platelets, lymphocyte count and monocyte count. These decreases were not seen or were less pronounced in the animals treated with either the C8.6.2 or Y61 antibody at 1 or 10 mg/kg (Groups 2-5).



Serum or plasma samples were analyzed by ELISA specific for monkey IFN-gamma and monkey IL-10 (Biosource International, Camarillo, CA), monkey IL-6 (Endogen) and plasma neopterin (ICN Pharmaceuticals, Orangeburg, NY). IFN-gamma, IL-10 or IL-6 were not detected in any of the IL-12 treated animals including the control  
5 animals treated with IL-12 plus IVIG. This was probably due to the low level exposure to IL-12 (only 1 dose of 1 µg/kg). Nevertheless, plasma neopterin levels increased about three fold in the IL-12 plus IVIG treated animals but did not change in all C8.6.2 or Y61 treated animals, including the lower dose (1 mg/kg) Y61 treated animals, indicating that Y61 was effective *in vivo* in blocking this sensitive response to IL-12.

10 In a second study, *in vivo* activity and pharmacodynamics (PD) of J695 in cynomolgous monkeys were studied by administering exogenous rhIL-12 and determining if J695 could block or reduce the responses normally associated with rhIL-12 administration. Male cynomolgus monkeys (n=3 per group) were administered a single dose of 0.05, 0.2, or 1.0 mg/kg J695 or 1 mg/kg intravenous immunoglobulin  
15 (IVIG) as a bolus intravenous (IV) injection via a saphenous vein or subcutaneously (SC) in the dorsal skin. One hour following the administration of J695 or IVIG, all animals received a single SC dose of 1 µg/kg rhIL-12 in the dorsal skin. Blood samples were collected via the femoral vein up to 28 days after J695 administration. Serum was acquired from each blood sample and assayed for IL-12, J695, IFN-γ, and anti-J695  
20 antibodies by ELISA. Neopterin was assayed by reverse-phase high performance liquid chromatography.

The levels of neopterin, normalized with respect to the levels of neopterin that were measured before administration of J695 or rhIL-12, are shown in Figure 3. To compare the suppression of neopterin between groups, the area under the curve (AUC)  
25 normalized for neopterin levels was calculated for each animal (Table 6). Neopterin exposure (AUC) was suppressed in a dose-dependent manner between approximately 71 and 93% in the IV groups and between 71 and 100% in SC groups, relative to the IVIG control groups. These results suggest that the dose of J695 necessary for 50% inhibition of the neopterin response (ED<sub>50</sub>) was less than 0.05 mg/kg when administered by either  
30 the IV or SC route.

Table 6: Dose-Dependent Suppression of IL-12 Induced Neopterin by J695 in Cynomolgus Monkeys

Route of dosing IVIG or J695 and rhIL-12	J695 Dose (mg/kg)	IVIG Dose (mg/kg)	AUC of Normalized Neopterin Levels	% Reduction of Neopterin AUC Compared with Control
Single IV injection followed 1 hr later by a dose of 1 µg/kg human IL-12 given SC	-	1.0	1745 ± 845	0
	0.05	-	502 ± 135	71.3
	0.2	-	199 ± 316	88.6
	1.0	-	128 ± 292	92.7
Single SC injection followed 1 hour later by a dose of 1 µg/kg human IL-12 given SC	-	1.0	1480 ± 604	0
	0.05	-	426 ± 108	71.2
	0.2	-	395 ± 45.9	73.3
	1.0	-	0 ± 109	100

5 Treatment with J695 also prevented or reduced the changes in hematology normally associated with rhIL-12 administration (leukopenia and thrombocytopenia). At 24 hours after rhIL-12 administration lymphocyte counts were reduced by approximately 50% when compared to baseline values in the control IV and SC IVIG treated groups. Administration of J695 either SC or IV at all three dose levels prevented  
 10 this reduction, resulting in lymphocyte counts at 24 hours approximately the same as baseline values. At 48 hours after IL-12 administration, platelet counts in the groups treated with IV and SC IVIG were reduced by approximately 25% when compared to baseline values.

An example dose schedule targeted to maintain serum levels above the 90%  
 15 effect level would be 1 mg/kg IV and SC given approximately every other week, or 0.3 mg/kg given approximately every week, assuming slight accumulation during repeated dosing. This study demonstrates that antibody can be given safely to monkeys at such dosages. In independent toxicity studies, it was further found that up to 100 mg/kg of the antibody can be given safely to monkeys.

20 J695 was also effective in preventing IFN-γ production in mice treated with a chimeric IL-12, a molecule which combines the murine p35 subunit with the human IL-12 p40 subunit. In contrast to human IL-12 which is biologically inactive in mice, this

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chimeric IL-12 retains biological function in mice, including induction of IFN- $\gamma$ . In addition, the human p40 subunit allows the molecule to be bound and neutralized by J695. Chimeric IL-12 at a dose of 0.05 mg/kg i.p. was administered to female C3H/HeJ mice (10/experimental group) in five daily doses on days 0, 1, 2, 3, and 4. J695 was  
5 given on days 0, 2 and 4 at doses of 0.05, 0.01, 0.002, 0.0004, 0.00008, and 0.000016 mg/kg i.p., 30' prior to the IL-12 injections. The control hulgG1 $\gamma$  was given IP. at a dose of 0.05 mg/kg on days 0, 2, and 4. The mice were bled on day 5, and serum IFN- $\gamma$  levels were determined by ELISA. The results demonstrated that J695 caused dose-dependent inhibition of IFN- $\gamma$  production with an ED<sub>50</sub> of approximately 0.001 mg/kg.  
10 Collectively, these results demonstrate that J695 is a potent inhibitor of IL-12 activity *in vivo*.

**EXAMPLE 5: Kinetic Analysis of Binding of Human Antibodies to Recombinant human IL-12 (rhIL-12)**

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Real-time binding interactions between captured ligand (human anti-rhIL-12 antibody J695, captured on a biosensor matrix) and analyte (rhIL12 in solution) were measured by surface plasmon resonance (SPR) using the BIAcore system (Biacore AB, Uppsala, Sweden). The system utilizes the optical properties of SPR to detect  
20 alterations in protein concentration within a dextran biosensor matrix. Proteins are covalently bound to the dextran matrix at known concentrations. Antibodies are injected through the dextran matrix and specific binding between injected antibodies and immobilized ligand results in an increased matrix protein concentration and resultant change in the SPR signal. These changes in SPR signal are recorded as resonance units  
25 (RU) and are displayed with respect to time along the y-axis of a sensorgram.

To facilitate immobilization of goat anti-human IgG (Southern Biotechnology Associates, Cat. No. 2040-01, Birmingham, AL) on the biosensor matrix, goat anti-human IgG is covalently linked via free amine groups to the dextran matrix by first activating carboxyl groups on the matrix with 100 mM N-hydroxysuccinimide (NHS)  
30 and 400 mM N-Ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC). Next, goat anti-human IgG is injected across the activated matrix. Thirty-five microliters of goat anti-human IgG (25  $\mu$ g/ml), diluted in sodium acetate, pH 4.5, is

injected across the activated biosensor and free amines on the protein are bound directly to the activated carboxyl groups. Unreacted matrix EDC-esters are deactivated by an injection of 1 M ethanolamine. Standard amine coupling kits were commercially available (Biacore AB, Cat. No. BR-1000-50. Uppsala. Sweden).

5 J695 was diluted in HBS running buffer (Biacore AB, Cat. No. BR-1001-88, Uppsala, Sweden) to be captured on the matrix via goat anti-human IgG. To determine the capacity of rhIL12-specific antibodies to bind immobilized goat anti-human IgG, a binding assay was conducted as follows. Aliquots of J695 (25 µg/ml; 25 µl aliquots) were injected through the goat anti-human IgG polyclonal antibody coupled dextran  
10 matrix at a flow rate of 5 µl/min. Before injection of the protein and immediately afterward, HBS buffer alone flowed through each flow cell. The net difference in signal between the baseline and the point corresponding to approximately 30 seconds after completion of J695 injection was taken to represent the amount of IgG1 J695 bound (approximately 1200 RU's). Direct rhIL12 specific antibody binding to soluble rhIL12  
15 was measured. Cytokines were diluted in HBS running buffer and 50 µl aliquots were injected through the immobilized protein matrices at a flow rate of 5 µl/min. The concentrations of rhIL-12 employed were 10, 20, 25, 40, 50, 80, 100, 150 and 200 nM. Prior to injection of rhIL-12, and immediately afterwards, HBS buffer alone flowed through each flow cell. The net difference in baseline signal and signal after completion  
20 of cytokine injection was taken to represent the binding value of the particular sample. Biosensor matrices were regenerated using 100 mM HCl before injection of the next sample. To determine the dissociation constant (off-rate), association constant (on-rate), BIAcore kinetic evaluation software (version 2.1) was used.

Representative results of CHO derived J695 binding to rhIL-12 as compared to  
25 the COS derived J695, are shown in Table 7.

Table 7: Binding of CHO or COS derived J695 to rhIL-12.

Source	rhIL12, nM	rhIL12 bound, RU's	Ab, bound, RU's	rhIL12/AB
CHO	200	1112	1613	1.48
CHO	150	1033	1525	1.45
CHO	100	994	1490	1.43

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CHO	80	955	1457	1.40
CHO	50	912	1434	1.36
CHO	40	877	1413	1.33
CHO	25	818	1398	1.25
CHO	20	773	1382	1.20
CHO	10	627	1371	0.98
Source	rhIL12, nM	rhIL12 bound, RU's	Ab, bound, RU's	rhIL12/AB
COS	200	1172	1690	1.49
COS	150	1084	1586	1.46
COS	100	1024	1524	1.44
COS	80	985	1489	1.42
COS	50	932	1457	1.37
COS	40	894	1431	1.34
COS	25	833	1409	1.27
COS	20	783	1394	1.20
COS	10	642	1377	1.00

Molecular kinetic interactions between captured J695 and soluble rhIL-12 were quantitatively analyzed using BIAcore technology. Several independent experiments were performed and the results were analyzed by the available BIAcore mathematical analysis software to derive kinetic rate constants, as shown in Table 8.

Table 8: Apparent kinetic rate and affinity constants of J695 for rhIL-12.

Antibody	Source	On-rate (M <sup>-1</sup> s <sup>-1</sup> ), Avg.	Off-rate (s <sup>-1</sup> ), Avg.	Kd (M), Avg.
J695	CHO	3.52E+05	4.72E-05	1.34E-10
J695	COS	3.40E+05	2.61E-05	9.74E-11

There was a small difference between the calculated apparent constant (Kd) for the interaction between CHO derived J695 ( $K_d = 1.34 \cdot 10^{-10} \text{M}^{-1}$ ) and COS derived J695 ( $K_d = 9.74 \times 10^{-11} \text{M}^{-1}$ ) antibodies. The apparent dissociation constant (Kd) between J695 and rhIL12 was estimated from the observed rate constants by the formula:  $K_d = \text{off-rate} / \text{on-rate}$ .

To determine the apparent association and dissociation rate constant for the interaction between J695 and rhIL-12, several binding reactions were performed using a fixed amount of J695 (2 µg/ml) and varying concentrations of rhIL-12. Real-time binding interaction sensorgrams between captured J695 and soluble rhIL12 showed that both forms of antibody were very similar for both the association and dissociation phase.

To further evaluate the capacity of captured IgG1 J695 mAb to bind soluble recombinant cytokine, a direct BIAcore method was used. In this method, goat anti-human IgG (25 µg/ml) coupled carboxymethyl dextran sensor surface was coated with IgG1 J695 (2µg/ml) and recombinant cytokine was then added. When soluble rhIL12 was injected across a biosensor surface captured with CHO or COS derived IgG1 J695, the amount of signal increased as the concentration of cytokine in the solution increased. No binding was observed with rmIL12 (R&D Systems, Cat. No. 419-ML, Minneapolis, MN) or rh IL12 any concentration tested up to 1000 nM. These results support the conclusion that IgG1 J695 antibodies recognize a distinct determinant on rhIL-12.

Table 9 shows the results of an experiment using BIAcore to demonstrate human IgG1 J695 mAb binding to only soluble rhIL12 and none of the other recombinant cytokines.

Table 9: Epitope mapping of J695 using BIAcore technology.

	Captured ligand COS J695	Captured ligand CHO J695
Soluble analyte		
rec. human IL12	Positive	Positive
rec. murine IL12	Negative	Negative

#### EXAMPLE 6: Further Studies of J695 Affinity for IL-12

Molecular kinetic interactions between J695 antibody and human IL-12 were quantitatively analyzed using BIAcore plasmon resonance technology, and apparent kinetic rate constants were derived.

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BIAcore technology was used to measure the binding of soluble rhIL-12 to solid phase captured J695. A goat anti-human IgG antibody was immobilized on the biosensor chips, then a fixed amount of J695 was injected and captured on the surface. Varying concentrations of rhIL-12 were applied, and the binding of IL-12 at different concentrations to J695 was measured as a function of time. Apparent dissociation and association rate constants were calculated, assuming zero-order dissociation and first order association kinetics, as well as a simple one-to-one molecular interaction between J695 and IL-12. Three independent experiments were performed, and the values shown are averages for the three experiments. From these measurements, the apparent dissociation ( $k_d$ ) and association ( $k_a$ ) rate constants were derived and used to calculate a  $K_d$  value for the interaction (see Table 10). The results indicated that J695 has a high affinity for rhIL-12.

Table 10: Kinetic Parameters for the Interaction Between J695 and Human IL-12

Kinetic Parameter	Value
$k_d$	$3.71 \pm 0.40 \times 10^{-5} \text{ s}^{-1}$
$k_a$	$3.81 \pm 0.48 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$
$K_d$	$9.74 \times 10^{-11} \text{ M (14 ng/mL)}$

**EXAMPLE 7: Characteristics and Neutralization Activity of C17.15, a Rat Monoclonal Antibody to Murine Interleukin-12**

To assess the relevance of IL-12 treatment studies in mouse models of inflammation and autoimmunity using monoclonal antibodies specific for murine IL-12 to similar approaches in human disease, the interaction of C17.15, a rat anti-murine IL-12 monoclonal antibody with murine IL-12, was examined. The ability of C17.15 to neutralize murine IL-12 activity in a PHA blast proliferation assay, and to block murine IL-12 binding to cell surface receptors, was assessed, as were the kinetics of the C17.15-murine IL-12 binding interaction.

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In a human PHA blast proliferation assay (See Example 3), serial dilutions of C17.15 or rat IgG2a (a control antibody) were preincubated with 230 pg/mL murine IL-12 for 1 hr at 37 °C. PHA-stimulated blast cells were added to the antibody-IL-12 mixtures and incubated for 3 days at 37 °C. The cells were subsequently labeled for 6 h with 1  $\mu$ Ci/well [ $^3$ H]-thymidine. The cultures were harvested and [ $^3$ H]-thymidine incorporation was measured. Background non-specific proliferation was measured in the absence of added murine IL-12. All samples were assayed in duplicate. The  $IC_{50}$  (M) of C17.15 for recombinant murine IL-12 in this assay was found to be  $1.4 \times 10^{-11}$ , as compared to the  $IC_{50}$  value of  $5.8 \times 10^{-12}$  observed for J695 for recombinant human IL-12 under the same conditions (see Table 11).

Table 11: Comparison of the properties of anti-human IL-12 monoclonal antibody J695 and the rat anti-mouse IL-12 monoclonal antibody C17.15

Antibody	Epitope	Biomolecular Interaction Assay			Receptor Binding Assay	PHA blast Assay
		$k_{on}$ , on-rate ( $M^{-1}s^{-1}$ )	$k_{off}$ , off-rate ( $s^{-1}$ )	$K_d$ (M)	$IC_{50}$ (M)	$IC_{50}$ (M)
J695	Hu p40	$3.81 \times 10^5$	$3.71 \times 10^{-3}$	$9.74 \times 10^{-11}$	$1.1 \times 10^{-11}$	$5.8 \times 10^{-12}$
C17.15	Mu p40	$3.80 \times 10^5$	$1.84 \times 10^{-4}$	$4.80 \times 10^{-10}$	$1.5 \times 10^{-10}$	$1.4 \times 10^{-11}$

The ability of C17.15 to inhibit the binding of murine IL-12 to cellular receptors was also measured. Serial dilutions of C17.15 were pre-incubated for 1 hr at 37 °C with 100 pM [ $^{125}$ I]-murine IL-12 in binding buffer. The 2D6 cells ( $2 \times 10^6$ ) were added to the antibody/[ $^{125}$ I]-murine IL-12 mixture and incubated for 2 hours at room temperature. Cell-bound radioactivity was separated from free [ $^{125}$ I]-IL-12, and the remaining cell-bound radioactivity was determined. Total binding of the labeled murine IL-12 to receptors on 2D6 cells was determined in the absence of antibody, and non-specific binding was determined by the inclusion of 25 nM unlabelled murine IL-12 in the assay. Specific binding was calculated as the total binding minus the non-specific binding. Incubations were carried out in duplicate. The results showed that C17.15 has an  $IC_{50}$  (M) of  $1.5 \times 10^{-10}$  for inhibition of binding of murine IL-12 to cellular receptors.



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The affinity of C17.15 for recombinant murine IL-12 was assessed by biomolecular interaction analysis. A goat anti-rat IgG antibody was immobilized on the biosensor chips, followed by an injection of a fixed amount of the C17.15 antibody, resulting in capture of C17.15 on the surface of the chip. Varying concentrations of recombinant murine IL-12 were applied to the C17.15 surface, and the binding of murine IL-12 to the immobilized C17.15 was measured as a function of time. Apparent dissociation and association rate constants were calculated, assuming a zero order dissociation and first order association kinetics as well as a simple one to one molecular interaction between the immobilized C17.15 and murine IL-12. From these measurements, the apparent dissociation ( $k_d$ , off-rate) and association ( $k_a$ , on-rate) rate constants were calculated. These results were used to calculate a  $K_d$  value for the interaction. An on-rate of  $3.8 \times 10^5 \text{ M}^{-1}\text{s}^{-1}$ , an off-rate of  $1.84 \times 10^{-4} \text{ s}^{-1}$ , and a  $K_d$  of  $4.8 \times 10^{-10}$  was observed for the recombinant murine IL-12-C17.15 interaction.

The observed activities of C17.15 in neutralizing murine IL-12 activity and binding to cell surface receptors, as well as the kinetics of binding of C17.15 to murine IL-12 correlate with similar measurements for the J695-rhIL-12 interaction. This indicates that the modes of action of the rat anti-mouse IL-12 antibody C17.15 and anti-human IL-12 antibody J695 are nearly identical based upon on-rate, off-rate,  $K_d$ ,  $\text{IC}_{50}$ , and the PHA blast assay. Therefore, C17.15 was used as a homologous antibody to J695 in murine models of inflammation and autoimmune disease to study the effects of IL-12 blockade on the initiation or progression of disease in these model animals (see Example 8).

**EXAMPLE 8: Treatment of Autoimmune or Inflammation-Based Diseases in Mice by  $\alpha$ -Murine IL-12 Antibody Administration**

**A. Suppression of Collagen-Induced Arthritis in Mice by the  $\alpha$ -IL-12 antibody C17.15**

A correlation between IL-12 levels and rheumatoid arthritis (RA) has been demonstrated. For example, elevated levels of IL-12 p70 have been detected in the synovia of RA patients compared with healthy controls (Morita et al (1998) *Arthritis and Rheumatism*. 41: 306-314). Therefore, the ability of C17.15, a rat anti-mouse IL-12 antibody, to suppress collagen-induced arthritis in mice was assessed.

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Male DBA/1 mice (10/group) were immunized with type II collagen on Day 0 and treated with C17.15, or control rat IgG, at 10 mg/kg intraperitoneally on alternate days from Day -1 (1 day prior to collagen immunization) to Day 12. The animals were monitored clinically for the development of arthritis in the paws until Day 90. The arthritis was graded as: 0- normal; 1- arthritis localized to one joint; 2- more than one joint involved but not whole paw; 3- whole paw involved; 4- deformity of paw; 5- ankylosis of involved joints. The arthritis score of a mouse was the sum of the arthritic grades in each individual paw of the mouse (max = 20). The results are expressed as mean  $\pm$  SEM in each group.

The results, as shown in Figure 4, indicate that an arthritic score was measurable in the C17.15-treated mice only after day 50 post-treatment, and that the peak mean arthritic score obtained with the C17.15-treated mice was at least 5-fold lower than that measured in the IgG-treated mice. This demonstrated that the rat anti-mouse IL-12 antibody C17.15 prevented the development of collagen-induced arthritis in mice.

#### B. Suppression of Colitis in Mice by the Rat $\alpha$ -Murine IL-12 Antibody C17.15

IL-12 has also been demonstrated to play a role in the development/pathology of colitis. For example, anti-IL-12 antibodies have been shown to suppress disease in mouse models of colitis, e.g., TNBS induced colitis IL-2 knockout mice (Simpson et al. (1998) *J. Exp. Med.* 187(8): 1225-34). Similarly, anti-IL-2 antibodies have been demonstrated to suppress colitis formation in IL-10 knock-out mice. The ability of the rat anti-mouse IL-12 antibody, C17.15, to suppress TNBS colitis in mice was assessed in two studies (Davidson et al. (1998) *J. Immunol.* 161(6): 3143-9).

In the first study, colitis was induced in pathogen free SJL mice by the administration of a 150  $\mu$ L 50% ethanol solution containing 2.0 mg TNBS delivered via a pediatric umbilical artery catheter into the rectum. Control animals were treated with a 150  $\mu$ L 50% ethanol solution only. A single dose of 0.75, 0.5, 0.25, or 0.1 mg C17.15 or 0.75 mg control rat IgG2a was given intravenously via the tail vein at day 11, and the therapeutic effect of the treatment was assessed by weighing the animals on days 11 and 17, and histological scoring at day 17. The weight of the mice treated with C17.15 increased within 48 hours of antibody treatment and normalized on day 6 after treatment. The effect of treatment with C17.15 was confirmed histologically. Further,

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assessments of IFN- $\gamma$  secretion by CD4<sup>+</sup> T-cells from spleen and colon of the treated mice, as well as IL-12 levels from spleen or colon-derived macrophages from the treated mice were also made (see Table 12).

In the second study, the dosing was optimized and the mice were treated with a total dose of 0.1 mg or 0.5 mg C17.15 or 0.1 mg control IgG2a, respectively, split between days 12 and 14. It was found that the administration of C17.15 in a single dose at the dosage of 0.1 mg/mouse or 0.25 mg/mouse led to only partial improvement in TNBS-induced colitis and did not result in a significant reduction in the CD4<sup>+</sup> T cell production of IFN- $\gamma$  *in vitro*, but did result in a significant decrease in secretion of IL-12, compared to untreated controls. At a single dose of 0.5 mg/mouse or greater a response was observed. Taking the lowest dose of antibody tested and administering it in two divided injections (at days 12 and 14) improved the dosing regimen, indicating that multiple low doses can be more effective than a single bolus dose. The data obtained are shown in Table 12.

Table 12: Anti-mouse IL-12 mAb C17.15 Suppresses Established Colitis in Mice

Disease Induction Day 0	Treatment Day 11	Weight (g)		IFN- $\gamma$ spleen CD4 <sup>+</sup> cells (U/mL)	IL-12 spleen macrophages (pg/ml)
		Day 11	Day 17		
TNBS + Ethanol	Control IgG2a 0.75 mg	16.0	15.26	3326	300
TNBS + Ethanol	C17.15 0.75 mg	16.0	20.21	1732	0
TNBS + Ethanol	C17.15 0.5 mg	16.36	19.94	1723	0
TNBS + Ethanol	C17.15 0.25 mg	16.28	17.7	3618	7
TNBS + Ethanol	C17.15 0.1 mg	16.2	17.98	3489	22
Ethanol control	-	20.76	21.16	1135	0

Administration of C17.15 monoclonal anti-IL-12 in two divided doses spaced one day apart totaling 0.1 mg/mouse or 0.05 mg/mouse led to complete reversal of colitis as assessed by wasting and macroscopic appearance of the colon. In addition, this dose schedule led to significant down-regulation of lamina propria T-cell production of IFN- $\gamma$  and macrophage production of IL-12, so that the latter were comparable to

levels seen in control ethanol-treated mice without TNBS-colitis. Thus, C17.15 administration to mouse models for TNBS colitis reversed the progression of the disease in a dose-dependent manner.

C. Suppression of Experimental Autoimmune Encephalomyelitis (EAE) in Mice by  $\alpha$ -

5 IL-12 Antibodies

It is commonly believed that IL-12 plays a role in the pathogenesis of multiple sclerosis (MS). The inducible IL-12 p40 message has been shown to be expressed in acute plaques of MS patients but not in inflammatory brain infarct lesions (Windhagen, A. *et al.* (1995) *J. Exp. Med.* 182: 1985-1996). T cells from MS patients (but not control  
10 T cells) stimulate IL-12 production from antigen-presenting cells through unregulated CD40L expression (Balashov, K.E. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94: 599-603). MS patients have enhanced IFN- $\gamma$  secretion that can be blocked with  $\alpha$ -IL-12 antibodies *in vitro* (Balashov, K.E. *et al.* (1997) *Proc. Natl. Acad. Sci. USA* 94: 599-603). Elevated levels of serum IL-12 are detected in MS patients, but not in other  
15 neurological diseases (Nicoletti, F. *et al.* (1996) *J. Neuroimmunol.* 70: 87-90). Increased IL-12 production has been shown to correlate with disease activity in MS patients (Cormabella, M. *et al.* (1998) *J. Clin. Invest.* 102: 671-678). The role of IL-12 in the pathogenesis of a murine model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), has been studied (Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181:  
20 281-386; Banerjee, S. *et al.* (1998) *Arthritis Rheum.* (1998) 41: S33; and Segal, B.M. *et al.* (1998) *J. Exp. Med.* 187: 537-546). The disease in this model is known to be induced by T cells of the TH<sub>1</sub> subset. Therefore, the ability of  $\alpha$ -IL-12 antibodies to prevent the onset of acute EAE was assessed.

An  $\alpha$ -IL-12 antibody was found to be able to inhibit the onset of acute EAE, to  
25 suppress the disease after onset, and to decrease the severity of relapses in mice immunized with the autoantigen, myelin basic protein (Banerjee, S. *et al.* (1998) *Arthritis Rheum.* (1998) 41: S33). The beneficial effects of  $\alpha$ -IL-12 antibody treatment in the mice persisted for over two months after stopping treatment. It has also been demonstrated that anti-IL-12 antibodies suppress the disease in mice that are recipients  
30 of encephalitogenic T cells by adoptive transfer (Leonard, J.P. *et al.* (1995) *J. Exp. Med.* 181: 281-386).

**EXAMPLE 9: Clinical Pharmacology of J695**

In a double blind, crossover study, 64 healthy, human male subjects were administered ascending doses of J695 or placebo. Measurement of complement fragment C3a prior to and 0.25 h after dosing did not demonstrate activation of the complement system. CRP and fibrinogen levels were only increased in subjects in whom symptoms of concurrent infections were observed.

All subjects survived and the overall tolerability of J695 was very good. In no case did treatment have to be stopped because of adverse events (AEs). The most commonly observed AEs were headache and common cold/bronchitis, neither of which were categorized as severe.

One of the study subjects, a 33-year-old single male, was suffering from psoriasis guttata at the start of the study. According to the randomized study design, this subject by chance received 5mg/kg J695 by SC administration. Ten days prior to administration of the antibody, the subject showed only small discrete papular lesions on the arms and legs. At the time of the antibody administration, the subject displayed increased reddening, thickness of the erythematous plaques, and increased hyperkeratosis. One week after J695 administration, the subject reported an improvement in skin condition, including flattening of the lesions and a decrease in scaling. Shortly after the second administration of J695 (5 mg/kg IV), the subject's skin was totally cleared of psoriatic lesions, in the absence of any local treatment. Erythematous plaques covered with white scales reappeared concomitant with the expected clearance of J695 after the second administration of antibody.

**EXAMPLE 10: Comparison of J695 Produced by Two CHO Cell Lines**

For recombinant expression of J695, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells (Urlaub, G. and Chasin, L.A. (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220) by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to enhancer/promoter regulatory elements (e.g., derived from SV40, CMV, adenovirus and

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the like, such as a CMV enhancer/AdMLP promoter regulatory element or an SV40 enhancer/AdMLP promoter regulatory element) to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification.

One hundred and fifty micrograms of an expression vector encoding the peptide sequences of the human antibody J695 were dissolved in 2.7 ml water in a 50 ml conical tube. Three hundred  $\mu$ L of 2.5 M  $\text{CaCl}_2$  were added and this DNA mixture was added dropwise to 3 ml of 2 x HEPES buffered saline in a 50 ml conical tube. After vortexing for 5 sec and incubating at room temperature for 20 min, 1 mL was distributed evenly over each plate (still in F12 medium), and the plates were incubated at 37 °C for 4 h. Liquid was removed by aspiration and 2 ml of 10% DMSO in F12 were added to each plate. The DMSO shock continued for 1 min, after which the DMSO was diluted by the addition of 5 ml PBS to each plate. Plates were washed twice in PBS, followed by the addition of 10 ml of alpha MEM, supplemented with H/T and 5% FBS (selective for cells expressing DHFR) and overnight incubation at 37 °C. Cells were seeded into 96-well plates at a density of 100 cells per well, and plates were incubated at 37 °C, 5%  $\text{CO}_2$  for two weeks, with one change of medium per week.

Five days after the final medium change, culture supernatants were diluted 1:50 and tested using an ELISA specific for human IgG gamma chain. The clones yielding the highest ELISA signal were transferred from the 96-well plates to 12-well plates in 1.5 ml/well of Alpha MEM + 5% dialyzed serum. After 3 days, another ELISA specific for human IgG gamma chain was performed, and the 12 clones with the greatest activity were split into the alpha MEM + 5% dialyzed serum and 20 nM MTX. Cell line 031898 218 grew in the presence of 20 nM MTX without any apparent cell death or reduction in growth rate, produced 1.8  $\mu$ g/ml hIgG in a three-day assay. T-25 cultures of 031898 218, growing in medium containing MTX, produced an average of 11.9  $\mu$ g/ml of J695. The line, designated ALP903, was adapted to growth in suspension under serum-free conditions, where it produced 7.5 pg J695/cell/24h.

ALP903 cells, after initial selection in alpha MEM/5% FBS/20 nM MTX medium, were passed again in 20 nM MTX. The cells were cultured under 100 nM MTX selection, followed by passaging in 500 nM MTX twice in the next 30 days. At

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that time the culture was producing 32  $\mu\text{g}$  J695/mL/24 h. The culture was subcloned by limiting dilution. Subclone 218-22 produced 16.5  $\mu\text{g}$ /mL in a 96-well plate in 2 days and 50.3  $\mu\text{g}$ /mL of J695 in a 12-well dish in 2 days. Clone 218-22 was cultured in alpha MEM/5% dialyzed FBS/500 nM MTX for 38 days, followed by adaptation to serum-free spinner culture, as above. The average cell-specific productivity of the serum-free suspension culture, designated ALP 905, was 58 pg/cell/24h.

The first cell line used to produce J695 (ALP 903) resulted in lower yields of the antibody from culture than a second cell line, ALP 905. To assure that the ALP 905-produced J695 was functionally identical to that produced from ALP 903, both batches of antibodies were assessed for IL-12 affinity, for the ability to block IL-12 binding to cellular receptors, for the ability to inhibit IFN- $\gamma$  induction by IL-12, and for the ability to inhibit IL-12-mediated PHA blast proliferation.

The affinities of J695 batches ALP 903 and ALP 905 for IL-12 were determined by measuring the kinetic rate constants of binding to IL-12 by surface plasmon resonance studies (BIAcore analyses). The off-rate constant ( $k_d$ ) and the on-rate constant ( $k_a$ ) of antibody batches ALP903 and ALP905 for binding to rhIL-12 were determined in three experiments (as described in Example 3). The affinity,  $K_d$ , of binding to IL-12 was calculated by dividing the off-rate constant by the on-rate constant.  $K_d$  was calculated for each separate experiment and then averaged. The results showed that the determined kinetic parameters and affinity of binding to rhIL-12 were very similar for J695 batches ALP 903 and ALP 905: the calculated  $K_d$  was  $1.19 \pm 0.22 \times 10^{-10}$  M for batch ALP 903 and  $1.49 \pm 0.47 \times 10^{-10}$  M for batch ALP 905 (see Table 13).

The ability of J695 derived from both ALP 903 and ALP 905 to block binding of rhIL-12 to IL-12 receptors on human PHA-activated T-lymphoblasts was assessed (see Example 3). Each sample of J695 was tested at a starting concentration of  $1 \times 10^{-8}$  with 10-fold serial dilutions. The antibody was preincubated for 1 hour at 37 °C with 50 pM [ $^{125}\text{I}$ ]-human IL-12 in binding buffer. PHA blast cells were added to the antibody/[ $^{125}\text{I}$ ]-human IL-12 mixture and incubated for 2 h at room temperature. Cell bound radioactivity was separated from free [ $^{125}\text{I}$ ]-IL-12 by centrifugation and washing steps, and % inhibition was calculated. The  $\text{IC}_{50}$  values for J695 were determined from the inhibition curves using 4-parameter curve fitting and were confirmed by two

EQUIVALENTS

Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention  
5 described herein. Such equivalents are intended to be encompassed by the following claims.



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Figure 1B. Heavy Chain Variable Region Sequences

SEQ ID NO:	Kabat number	CDR H2	CDR H3	
33	JOE9wt VH	KYYADSVKG	SGSYDY	WGQGTMTVSS
35	Cos-3/JH3 VH	.....	H..H.N	.....
37	70-1 VH	.....	.....	.....
39	78-34 VH	.....	.....	.....
41	79-1 VH	.....	.....	.....
43	101-11 VH	.....	H..H.N	.....
45	26-1 VH	.....	H..H.N	.....
47	136-15 VH	.....	H..H.N	.....
49	136-15 VH germline	.....	H..H.N	.....
51	149-5 VH	.....	H..H.T	.....
53	149-6 VH	.....	H..H.N	.....
55	103-4 VH	.....	H..H.N	.....
57	103-8 VH	.....	H..H.N	.....
59	103-14 VH	.....	H..H.N	.....
61	G6 VH	.....	H..H.N	.....
63	Y139 VH	.....	H..H.N	.....
65	AO3 VH	.....	H..H.N	.....
67	AO3 VH germline	.....	H..H.N	.....
23	Y61 VH	.....	H..H.N	.....
69	Y61 VH germline	.....	H..H.N	.....
71	Y61-H31E VH	.....	H..H.N	.....
73	Y61 L50Y VH	.....	H..H.N	.....
75	Y61-L94Y VH	.....	H..H.N	.....
31	J695	.....	H..H.N	.....

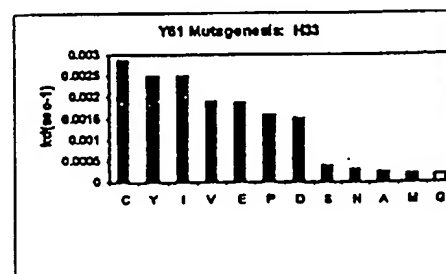
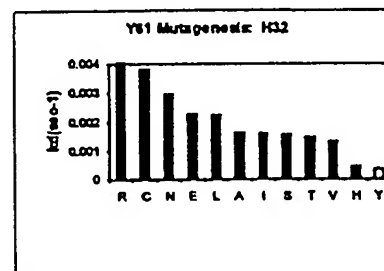
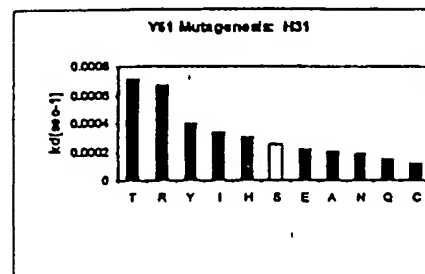
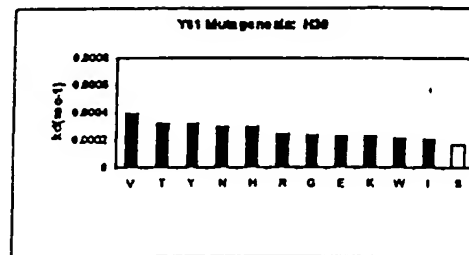




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Figure 2A. Y61 Heavy Chain CDR H1 Mutagenesis

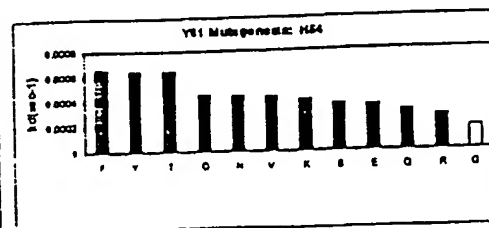
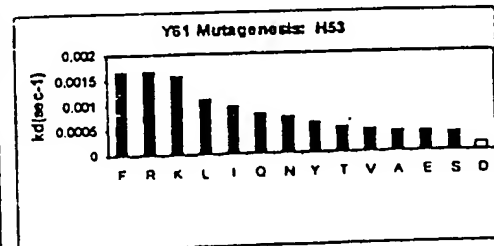
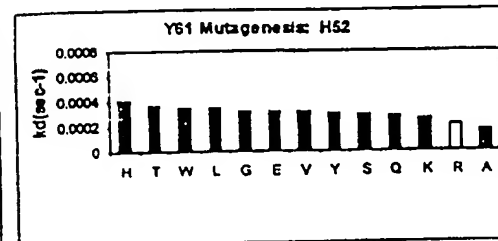
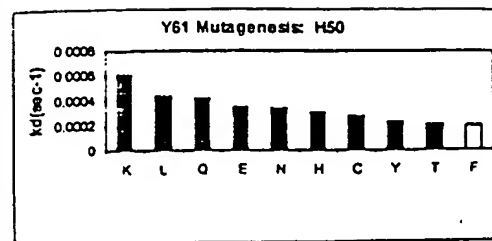
SEQ ID NO:		CDR H1									$k_{off}$ ( $\times 10^5$ )
		27	28	29	30	31	32	33	34	35	
21	Y61	F	T	F	S	S	Y	G	M	H	
288		.	.	.	E	.	.	.	.	.	22.8
289		.	.	.	S	.	.	.	.	.	16.8
290		.	.	.	Y	.	.	.	.	.	31.9
291		.	.	.	H	.	.	.	.	.	29.6
292		.	.	.	K	.	.	.	.	.	22.5
293		.	.	.	R	.	.	.	.	.	24.5
294		.	.	.	N	.	.	.	.	.	30.1
295		.	.	.	T	.	.	.	.	.	32.0
296		.	.	.	G	.	.	.	.	.	23.3
297		.	.	.	V	.	.	.	.	.	39.9
298		.	.	.	I	.	.	.	.	.	20.7
299		.	.	.	W	.	.	.	.	.	21.6
300		.	.	.	E	.	.	.	.	.	21.9
301		.	.	.	C	.	.	.	.	.	12.0
302		.	.	.	S	.	.	.	.	.	24.9
303		.	.	.	Y	.	.	.	.	.	39.8
304		.	.	.	H	.	.	.	.	.	30.9
305		.	.	.	R	.	.	.	.	.	66.4
306		.	.	.	N	.	.	.	.	.	19.1
307		.	.	.	Q	.	.	.	.	.	15.2
308		.	.	.	T	.	.	.	.	.	71.6
309		.	.	.	A	.	.	.	.	.	20.5
310		.	.	.	I	.	.	.	.	.	33.4
311		.	.	.	E	.	.	.	.	.	229.0
312		.	.	.	C	.	.	.	.	.	383.0
313		.	.	.	S	.	.	.	.	.	157.5
314		.	.	.	Y	.	.	.	.	.	33.7
315		.	.	.	H	.	.	.	.	.	46.1
316		.	.	.	R	.	.	.	.	.	448.5
317		.	.	.	N	.	.	.	.	.	297.0
318		.	.	.	T	.	.	.	.	.	148.0
319		.	.	.	A	.	.	.	.	.	165.5
320		.	.	.	V	.	.	.	.	.	133.5
321		.	.	.	L	.	.	.	.	.	226.0
322		.	.	.	I	.	.	.	.	.	160.5
323		.	.	.	D	.	.	.	.	.	152.0
324		.	.	.	E	.	.	.	.	.	189.0
325		.	.	.	C	.	.	.	.	.	286.5
326		.	.	.	S	.	.	.	.	.	39.9
327		.	.	.	Y	.	.	.	.	.	250.5
328		.	.	.	N	.	.	.	.	.	30.8
329		.	.	.	G	.	.	.	.	.	17.8
330		.	.	.	A	.	.	.	.	.	27.3
331		.	.	.	V	.	.	.	.	.	191.0
332		.	.	.	M	.	.	.	.	.	21.5
333		.	.	.	I	.	.	.	.	.	250.0
334		.	.	.	P	.	.	.	.	.	159.5



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Figure 2B. Y61 Heavy Chain CDR H2 Mutagenesis

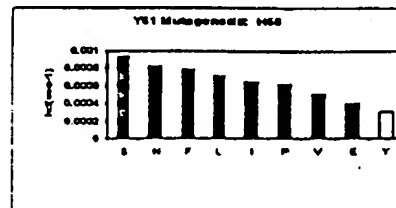
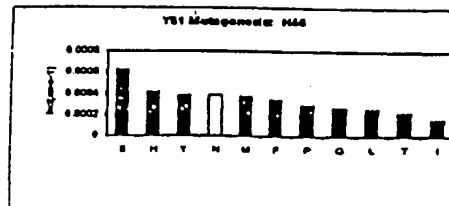
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19	Y61	F	I	R	Y	D	G	S	N	K	Y	Y	A	D	S	V	K	G
335		E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34.7
336		C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	28.5
337		Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	23.0
338		H	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	30.9
339		K	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	61.2
340		N	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34.4
341		Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	42.0
342		T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20.5
343		L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	44.0
344		F	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20.4
345		.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	.	31.8
346		.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	.	29.2
347		.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	.	29.8
348		.	H	.	.	.	.	.	.	.	.	.	.	.	.	.	.	40.7
349		.	K	.	.	.	.	.	.	.	.	.	.	.	.	.	.	26.2
350		.	R	.	.	.	.	.	.	.	.	.	.	.	.	.	.	20.6
351		.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	.	28.5
352		.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	37.4
353		.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	32.1
354		.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	17.1
355		.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	.	31.7
356		.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	.	34.7
357		.	W	.	.	.	.	.	.	.	.	.	.	.	.	.	.	35.1
358		.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	15.1
359		.	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	39.9
360		.	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	36.8
361		.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	61.1
362		.	.	K	.	.	.	.	.	.	.	.	.	.	.	.	.	158.0
363		.	.	R	.	.	.	.	.	.	.	.	.	.	.	.	.	166.5
364		.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	72.7
365		.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	79.2
366		.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	50.0
367		.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	40.4
368		.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	44.0
369		.	.	L	.	.	.	.	.	.	.	.	.	.	.	.	.	109.5
370		.	.	I	.	.	.	.	.	.	.	.	.	.	.	.	.	94.4
371		.	.	F	.	.	.	.	.	.	.	.	.	.	.	.	.	168.5
372		.	.	D	.	.	.	.	.	.	.	.	.	.	.	.	.	45.5
373		.	.	E	.	.	.	.	.	.	.	.	.	.	.	.	.	35.1
374		.	.	S	.	.	.	.	.	.	.	.	.	.	.	.	.	37.3
375		.	.	Y	.	.	.	.	.	.	.	.	.	.	.	.	.	64.6
376		.	.	K	.	.	.	.	.	.	.	.	.	.	.	.	.	40.7
377		.	.	R	.	.	.	.	.	.	.	.	.	.	.	.	.	2.5
378		.	.	N	.	.	.	.	.	.	.	.	.	.	.	.	.	44.7
379		.	.	Q	.	.	.	.	.	.	.	.	.	.	.	.	.	31.6
380		.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	64.4
381		.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	17.8
382		.	.	V	.	.	.	.	.	.	.	.	.	.	.	.	.	43.5



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Figure 2C. Y61 Heavy Chain CDR H2 Mutagenesis

SEQ ID NO:		CDR H2																	$k_{eff}$ ( $\times 10^3$ )
		50	51	52	52A	53	54	55	56	57	58	59	60	61	62	63	64	65	
19	Y61	F	I	R	Y	D	G	S	N	K	Y	Y	A	D	S	V	K	G	
383		.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	.	66.3
384		.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	.	62.4
385		.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	.	39.0
386		.	.	.	.	.	.	H	.	.	.	.	.	.	.	.	.	.	42.0
387		.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	.	38.5
388		.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	23.5
389		.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	27.2
390		.	.	.	.	.	.	M	.	.	.	.	.	.	.	.	.	.	38.3
391		.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	.	26.4
392		.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	.	16.9
393		.	.	.	.	.	.	P	.	.	.	.	.	.	.	.	.	.	29.9
394		.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	.	34.5
395		.	.	.	.	.	.	.	E	.	.	.	.	.	.	.	.	.	41.5
396		.	.	.	.	.	.	.	S	.	.	.	.	.	.	.	.	.	94.1
397		.	.	.	.	.	.	.	Y	.	.	.	.	.	.	.	.	.	31.0
398		.	.	.	.	.	.	.	N	.	.	.	.	.	.	.	.	.	83.1
399		.	.	.	.	.	.	.	V	.	.	.	.	.	.	.	.	.	52.4
400		.	.	.	.	.	.	.	L	.	.	.	.	.	.	.	.	.	73.0
401		.	.	.	.	.	.	.	I	.	.	.	.	.	.	.	.	.	65.7
402		.	.	.	.	.	.	.	P	.	.	.	.	.	.	.	.	.	62.8
403		.	.	.	.	.	.	.	F	.	.	.	.	.	.	.	.	.	79.4



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Figure 2D. Y61 Heavy Chain CDR H3 Mutagenesis

SEQ ID NO:		CDR H3						$K_{off}$ ( $\times 10^3$ )
		95	96	97	98	101	102	
17	Y61	H	G	S	H	D	N	
404		E	.	.	.	.	.	231.5
405		S	.	.	.	.	.	193.0
406		H	.	.	.	.	.	28.7
407		K	.	.	.	.	.	227.5
408		Q	.	.	.	.	.	85.9
409		T	.	.	.	.	.	202.0
410		A	.	.	.	.	.	150.0
411		L	.	.	.	.	.	147.5
412		P	.	.	.	.	.	471.0
413		F	.	.	.	.	.	514.0
414		.	D	.	.	.	.	223.5
415		.	C	.	.	.	.	24.2
416		.	H	.	.	.	.	23.7
417		.	R	.	.	.	.	96.2
418		.	T	.	.	.	.	186.0
419		.	G	.	.	.	.	39.7
420		.	V	.	.	.	.	38.2
421		.	M	.	.	.	.	204.5
422		.	L	.	.	.	.	261.0
423		.	I	.	.	.	.	207.5
424		.	P	.	.	.	.	129.0
425		.	W	.	.	.	.	197.0
426		.	.	D	.	.	.	202.0
427		.	.	S	.	.	.	37.5
428		.	.	Y	.	.	.	273.0
429		.	.	H	.	.	.	190.5
430		.	.	R	.	.	.	224.0
431		.	.	N	.	.	.	221.5
432		.	.	T	.	.	.	58.8
433		.	.	G	.	.	.	229.0
434		.	.	A	.	.	.	143.0
435		.	.	I	.	.	.	208.0
436		.	.	P	.	.	.	300.0
437		.	.	W	.	.	.	239.0
438		.	.	F	.	.	.	180.5
439		.	.	.	H	.	.	25.5
440		.	.	.	R	.	.	34.0
441		.	.	.	T	.	.	22.7
442		.	.	.	A	.	.	67.3
443		.	.	.	V	.	.	29.3
444		.	.	.	L	.	.	59.8
445		.	.	.	I	.	.	34.3
446		.	.	.	F	.	.	68.8
447		.	.	.	.	D	.	14.4
448		.	.	.	.	S	.	44.9
449		.	.	.	.	Y	.	465.0
450		.	.	.	.	H	.	327.0
451		.	.	.	.	R	.	110.0

Y61 Mutagenesis: H95



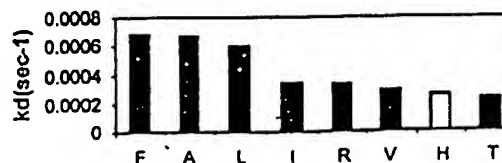
Y61 Mutagenesis: H96



Y61 Mutagenesis: H97



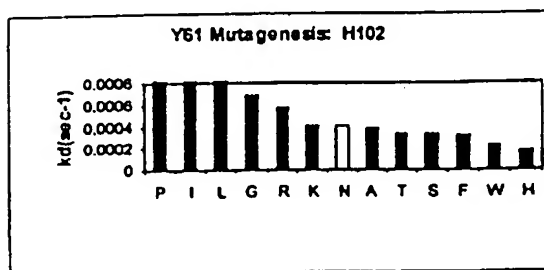
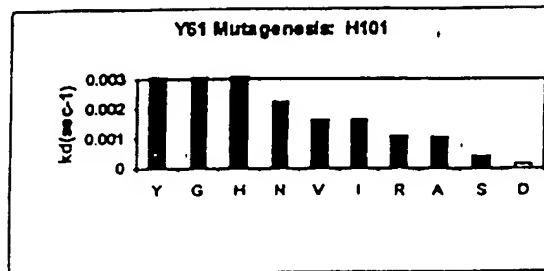
Y61 Mutagenesis: H98



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Figure 2E. Y61 Heavy Chain CDR H3 Mutagenesis

SEQ ID NO:		CDR H3						$K_{off}$ ( $\times 10^3$ )
		95	96	97	98	101	102	
17	Y61	H	G	S	H	D	N	
452		.	.	.	.	N	.	223.0
453		.	.	.	.	G	.	375.0
454		.	.	.	.	A	.	106.5
455		.	.	.	.	V	.	163.0
456		.	.	.	.	I	.	162.5
457		.	.	.	.	.	S	32.5
458		.	.	.	.	.	H	18.0
459		.	.	.	.	.	K	40.5
460		.	.	.	.	.	R	57.5
461		.	.	.	.	.	N	40.3
462		.	.	.	.	.	T	33.3
463		.	.	.	.	.	G	69.2
464		.	.	.	.	.	A	38.2
465		.	.	.	.	.	L	95.6
466		.	.	.	.	.	I	99.6
467		.	.	.	.	.	P	181.5
468		.	.	.	.	.	W	23.5
469		.	.	.	.	.	F	31.8

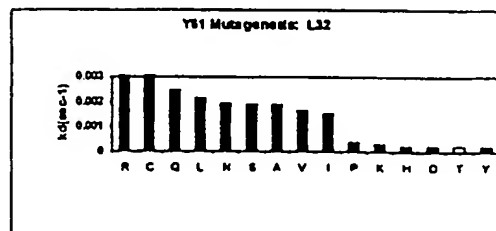
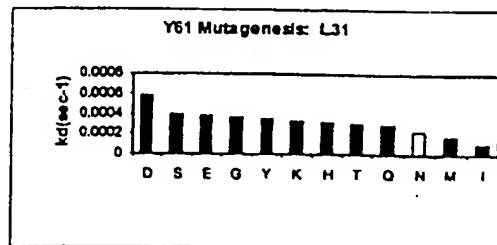
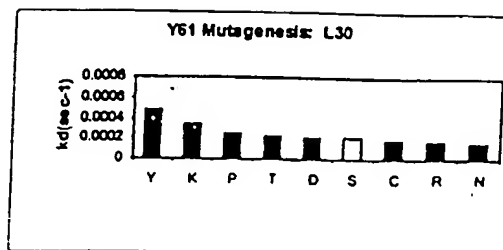




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Figure 2F. Y61 Light Chain CDR L1 Mutagenesis

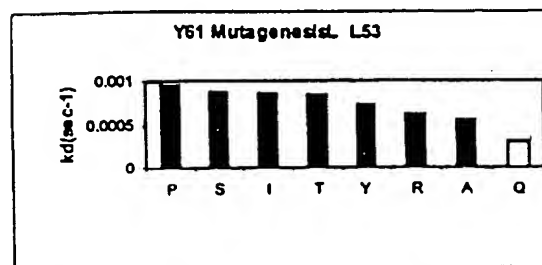
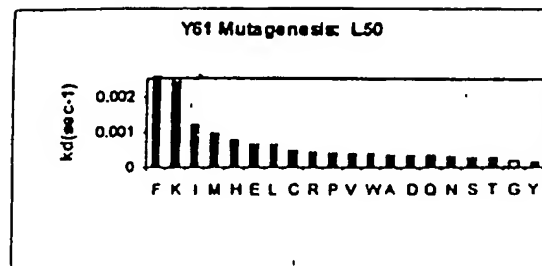
SEQ ID NO:	CDR L1													$k_{orr}$ ( $\times 10^3$ )	
	24	25	26	27	27A	27B	28	29	30	31	32	33	34		
22	Y61	S	G	G	R	S	N	I	G	S	N	T	V	K	
470		.	.	.	.	.	.	.	.	D	.	.	.	.	22.0
471		.	.	.	.	.	.	.	.	C	.	.	.	.	18.6
472		.	.	.	.	.	.	.	.	S	.	.	.	.	21.1
473		.	.	.	.	.	.	.	.	Y	.	.	.	.	48.3
474		.	.	.	.	.	.	.	.	K	.	.	.	.	34.6
475		.	.	.	.	.	.	.	.	R	.	.	.	.	18.2
476		.	.	.	.	.	.	.	.	N	.	.	.	.	16.6
477		.	.	.	.	.	.	.	.	T	.	.	.	.	22.6
478		.	.	.	.	.	.	.	.	P	.	.	.	.	25.0
479		.	.	.	.	.	.	.	.	D	.	.	.	.	58.0
480		.	.	.	.	.	.	.	.	E	.	.	.	.	38.4
481		.	.	.	.	.	.	.	.	S	.	.	.	.	39.2
482		.	.	.	.	.	.	.	.	Y	.	.	.	.	35.7
483		.	.	.	.	.	.	.	.	H	.	.	.	.	31.5
484		.	.	.	.	.	.	.	.	K	.	.	.	.	33.1
485		.	.	.	.	.	.	.	.	N	.	.	.	.	22.9
486		.	.	.	.	.	.	.	.	Q	.	.	.	.	29.2
487		.	.	.	.	.	.	.	.	T	.	.	.	.	30.9
488		.	.	.	.	.	.	.	.	G	.	.	.	.	36.6
489		.	.	.	.	.	.	.	.	M	.	.	.	.	17.4
490		.	.	.	.	.	.	.	.	I	.	.	.	.	9.7
491		.	.	.	.	.	.	.	.	D	.	.	.	.	25.2
492		.	.	.	.	.	.	.	.	C	.	.	.	.	381.5
493		.	.	.	.	.	.	.	.	S	.	.	.	.	191.0
494		.	.	.	.	.	.	.	.	Y	.	.	.	.	21.3
495		.	.	.	.	.	.	.	.	H	.	.	.	.	26.0
496		.	.	.	.	.	.	.	.	K	.	.	.	.	31.8
497		.	.	.	.	.	.	.	.	R	.	.	.	.	690.0
498		.	.	.	.	.	.	.	.	N	.	.	.	.	196.5
499		.	.	.	.	.	.	.	.	Q	.	.	.	.	247.0
500		.	.	.	.	.	.	.	.	T	.	.	.	.	24.1
501		.	.	.	.	.	.	.	.	A	.	.	.	.	190.5
502		.	.	.	.	.	.	.	.	V	.	.	.	.	164.5
503		.	.	.	.	.	.	.	.	L	.	.	.	.	215.5
504		.	.	.	.	.	.	.	.	I	.	.	.	.	154.0
505		.	.	.	.	.	.	.	.	P	.	.	.	.	42.4



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Figure 2G. Y61 Light Chain CDR L2 Mutagenesis

SEQ ID NO:		CDR L2							$K_{off}$ ( $\times 10^4$ )
		S0	S1	S2	S3	S4	S5	S6	
20	Y61	G	N	D	Q	R	P	S	
506		D	.	.	.	.	.	.	34.8
507		E	.	.	.	.	.	.	61.7
508		C	.	.	.	.	.	.	46.7
509		S	.	.	.	.	.	.	28.6
510		Y	.	.	.	.	.	.	17.4
511		H	.	.	.	.	.	.	76.1
512		K	.	.	.	.	.	.	242.5
513		R	.	.	.	.	.	.	44.4
514		N	.	.	.	.	.	.	30.5
515		Q	.	.	.	.	.	.	34.8
516		T	.	.	.	.	.	.	27.2
517		G	.	.	.	.	.	.	21.5
518		A	.	.	.	.	.	.	37.2
519		V	.	.	.	.	.	.	38.5
520		M	.	.	.	.	.	.	95.3
521		L	.	.	.	.	.	.	61.6
522		I	.	.	.	.	.	.	120.5
523		P	.	.	.	.	.	.	41.0
524		W	.	.	.	.	.	.	38.2
525		F	.	.	.	.	.	.	3,476.7
526		.	.	.	S	.	.	.	86.6
527		.	.	.	Y	.	.	.	73.3
528		.	.	.	R	.	.	.	61.4
529		.	.	.	Q	.	.	.	29.7
530		.	.	.	T	.	.	.	83.4
531		.	.	.	A	.	.	.	55.4
532		.	.	.	I	.	.	.	85.5
533		.	.	.	P	.	.	.	97.4



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Figure 2H. Y61 Light Chain CDR L3 Mutagenesis

SEQ ID NO:		CDR L3												$k_{off}$ ( $\times 10^3$ )
		89	90	91	92	93	94	95	95A	95B	95C	96	97	
18	Y61	Q	S	Y	D	R	G	T	H	P	A	L	L	
534		.	.	.	D	.	.	.	.	.	.	.	.	25.9
535		.	.	.	C	.	.	.	.	.	.	.	.	45.3
536		.	.	.	S	.	.	.	.	.	.	.	.	30.7
537		.	.	.	Y	.	.	.	.	.	.	.	.	51.1
538		.	.	.	N	.	.	.	.	.	.	.	.	34.7
539		.	.	.	Q	.	.	.	.	.	.	.	.	42.7
540		.	.	.	T	.	.	.	.	.	.	.	.	40.8
541		.	.	.	G	.	.	.	.	.	.	.	.	34.9
542		.	.	.	A	.	.	.	.	.	.	.	.	35.7
543		.	.	.	L	.	.	.	.	.	.	.	.	72.8
544		.	.	.	I	.	.	.	.	.	.	.	.	61.8
545		.	.	.	W	.	.	.	.	.	.	.	.	72.0
546		.	.	.	F	.	.	.	.	.	.	.	.	44.9
547		.	.	.	D	.	.	.	.	.	.	.	.	34.3
548		.	.	.	C	.	.	.	.	.	.	.	.	32.0
549		.	.	.	S	.	.	.	.	.	.	.	.	34.1
550		.	.	.	Y	.	.	.	.	.	.	.	.	33.5
551		.	.	.	R	.	.	.	.	.	.	.	.	19.9
552		.	.	.	N	.	.	.	.	.	.	.	.	31.6
553		.	.	.	Q	.	.	.	.	.	.	.	.	30.0
554		.	.	.	T	.	.	.	.	.	.	.	.	31.6
555		.	.	.	G	.	.	.	.	.	.	.	.	39.2
556		.	.	.	A	.	.	.	.	.	.	.	.	31.0
557		.	.	.	V	.	.	.	.	.	.	.	.	26.9
558		.	.	.	M	.	.	.	.	.	.	.	.	27.5
559		.	.	.	L	.	.	.	.	.	.	.	.	30.0
560		.	.	.	I	.	.	.	.	.	.	.	.	29.5
561		.	.	.	P	.	.	.	.	.	.	.	.	34.9
562		.	.	.	W	.	.	.	.	.	.	.	.	34.9
563		.	.	.	D	.	.	.	.	.	.	.	.	25.3
564		.	.	.	C	.	.	.	.	.	.	.	.	52.0
565		.	.	.	S	.	.	.	.	.	.	.	.	28.7
566		.	.	.	Y	.	.	.	.	.	.	.	.	13.1
567		.	.	.	H	.	.	.	.	.	.	.	.	18.7
568		.	.	.	R	.	.	.	.	.	.	.	.	23.1
569		.	.	.	N	.	.	.	.	.	.	.	.	13.7
570		.	.	.	Q	.	.	.	.	.	.	.	.	25.0
571		.	.	.	T	.	.	.	.	.	.	.	.	30.5
572		.	.	.	G	.	.	.	.	.	.	.	.	25.6
573		.	.	.	A	.	.	.	.	.	.	.	.	52.6
574		.	.	.	V	.	.	.	.	.	.	.	.	35.1
575		.	.	.	L	.	.	.	.	.	.	.	.	24.4
576		.	.	.	I	.	.	.	.	.	.	.	.	27.6
577		.	.	.	P	.	.	.	.	.	.	.	.	33.2
578		.	.	.	W	.	.	.	.	.	.	.	.	29.3
579		.	.	.	F	.	.	.	.	.	.	.	.	23.6

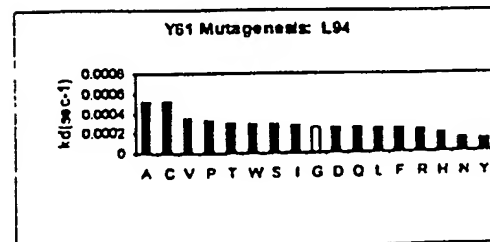
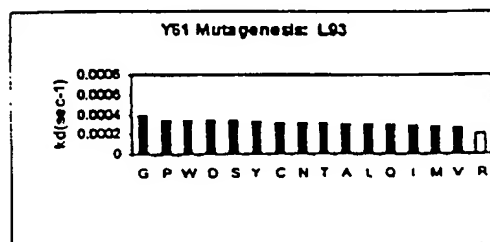
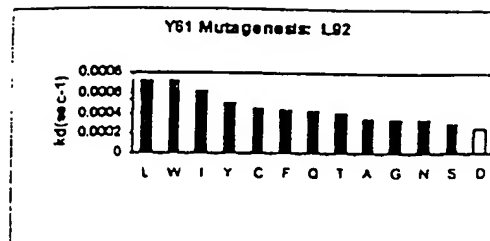
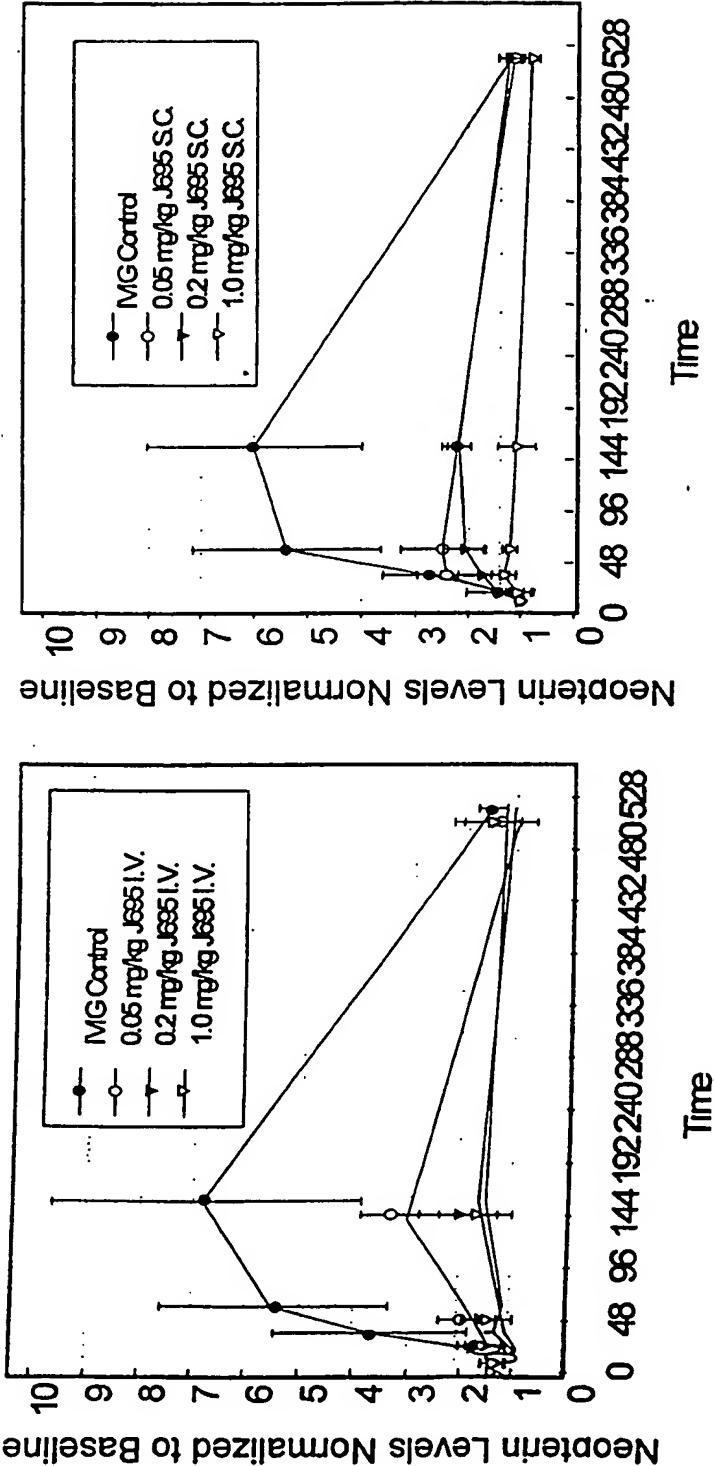


Figure 3: *In vivo* efficacy of J695 in cynomolgus monkeys



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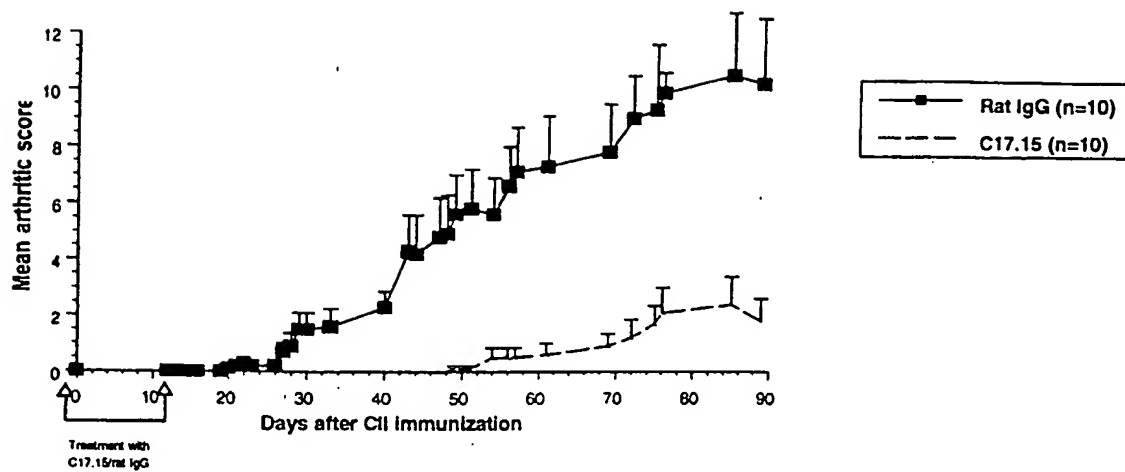


FIGURE 4

- 1 -

## SEQUENCE LISTING

<110> Jochen, Salfeld et al.

<120> Human Antibodies That Bind Human IL-12 And Methods For Producing

<130> BBI-093CPPC

<140>

<141>

<150> 60/126,603

<151> March 25, 1999

<160> 675

<170> PatentIn Ver. 2.0

<210> 1

<211> 6

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 1 could be either His or Ser

<220>

<223> Xaa at position 4 could be either Tyr or His

<220>

<223> Xaa at position 6 could be either Tyr, Asn or Thr

<400> 1

Xaa Gly Ser Xaa Asp Xaa  
1 5

<210> 2

<211> 12

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<213> Homo sapiens

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<223> Xaa at position 2 could be either Ser or Thr

<220>

<223> Xaa at position 4 could be either Asp or Glu

<220>

<223> Xaa at position 5 could be either Ser, Arg or Lys

<220>

<223> Xaa at position 6 could be either Ser, Gly or Tyr

<220>

<223> Xaa at position 7 could be either Leu, Phe, Thr or Ser

<220>

<223> Xaa at position 8 could be either Arg, Ser, Thr, Trp or His

<220>

<223> Xaa at position 9 could be either Gly or Pro

<220>

- 2 -

<223> Xaa at position 10 could be either Ser, Thr, Ala  
or Leu

<220>

<223> Xaa at position 11 could be either Arg, Ser, Met,  
Thr or Leu

<220>

<223> Xaa at position 12 could be either Val, Ile, Thr,  
Met or Leu

<400> 2

Gln Xaa Tyr Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa  
1 5 10

<210> 3

<211> 17

<212> PRT

<213> Homo sapiens

<400> 3

Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys  
1 5 10 15

Gly

<210> 4

<211> 7

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa at position 1 could be either Gly or Tyr

<220>

<223> Xaa at position 3 could be either Asp or Ser

<220>

<223> Xaa at position 4 could be either Gln or Asn

<400> 4

Xaa Asn Xaa Xaa Arg Pro Ser  
1 5

<210> 5

<211> 9

<212> PRT

<213> Homo sapiens

<220>

<223> Xaa represents either Ser or Glu

<400> 5

Phe Thr Phe Ser Xaa Tyr Gly Met His  
1 5

<210> 6

<211> 13

<212> PRT

<213> Homo sapiens

- 3 -

&lt;220&gt;

&lt;223&gt; Xaa at position 1 could be either Ser or Thr

&lt;220&gt;

&lt;223&gt; Xaa at position 3 could be either Ser or Gly

&lt;220&gt;

&lt;223&gt; Xaa at position 4 could be either Arg or Ser

&lt;220&gt;

&lt;223&gt; Xaa at position 8 could be either Gly or Val

&lt;220&gt;

&lt;223&gt; Xaa at position 9 could be either Ser or Ala

&lt;220&gt;

&lt;223&gt; Xaa at position 10 could be either Asn, Gly or Tyr

&lt;220&gt;

&lt;223&gt; Xaa at position 11 could be either Thr or Asp

&lt;220&gt;

&lt;223&gt; Xaa at position 13 could be either Lys or His

&lt;400&gt; 6

Xaa	Gly	Xaa	Xaa	Ser	Asn	Ile	Xaa	Xaa	Xaa	Val	Xaa
1				5						10	

&lt;210&gt; 7

&lt;211&gt; 115

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Xaa at position 6 could be either Gln or Glu

&lt;220&gt;

&lt;223&gt; Xaa at position 16 could be either Arg or Gly

&lt;220&gt;

&lt;223&gt; Xaa at position 31 could be either Ser or Glu

&lt;220&gt;

&lt;223&gt; Xaa at position 84 could be either Lys or Asn

&lt;220&gt;

&lt;223&gt; Xaa at position 97 could be either Thr, Ala or Lys

&lt;220&gt;

&lt;223&gt; Xaa at position 98 could be either Thr or Lys

&lt;220&gt;

&lt;223&gt; Xaa at position 99 could be either Ser or His

&lt;220&gt;

&lt;223&gt; Xaa at position 102 could be either Tyr or His

&lt;220&gt;

&lt;223&gt; Xaa at position 104 could be either Tyr, Asn or Thr

&lt;400&gt; 7

Gln	Val	Gln	Leu	Val	Xaa	Ser	Gly	Gly	Gly	Val	Val	Gln	Pro	Gly	Xaa
1				5					10					15	



- 4 -

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Xaa Tyr  
                   20                                  25                                  30  
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
                   35                                  40                                  45  
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Asx  
                   50                                  55                                  60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
                   65                                  70                                  75                                  80  
 Leu Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
                                   85                                  90                                  95  
 Xaa Xaa Xaa Gly Ser Xaa Asp Xaa Trp Gly Gln Gly Thr Met Val Thr  
                   100                                  105                                  110  
 Val Ser Ser  
                   115

&lt;210&gt; 8

&lt;211&gt; 112

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Xaa at position 1 could be either Ser or Gln

&lt;220&gt;

&lt;223&gt; Xaa at position 2 could be either Tyr or Ser

&lt;220&gt;

&lt;223&gt; Xaa at position 13 could be either Thr or Ala

&lt;220&gt;

&lt;223&gt; Xaa at position 23 and 91 could be either Ser or Thr

&lt;220&gt;

&lt;223&gt; Xaa at position 25 could be either Gly or Ser

&lt;220&gt;

&lt;223&gt; Xaa at position 26 could be either Arg or Ser

&lt;220&gt;

&lt;223&gt; Xaa at position 30 could be either Gly or Val

&lt;220&gt;

&lt;223&gt; Xaa at position 31 could be either Ser or Ala

&lt;220&gt;

&lt;223&gt; Xaa at position 35 could be either Lys or His

&lt;220&gt;

&lt;223&gt; Xaa at position 51 could be either Gly or Lys

&lt;220&gt;

&lt;223&gt; Xaa at position 54 could be either Gln or Asn

&lt;220&gt;

&lt;223&gt; Xaa at position 79 could be either Val or Leu

&lt;220&gt;

&lt;223&gt; Xaa at position 93 could be either Asp or Glu

- 5 -

&lt;220&gt;

&lt;223&gt; Xaa at position 94 could be either Ser, Arg or Lys

&lt;220&gt;

&lt;223&gt; Xaa at position 95 could be either Ser, Gly or Tyr

&lt;220&gt;

<223> Xaa at position 96 could be either Leu, Phe, Thr  
or Ser

&lt;220&gt;

<223> Xaa at position 97 could be either Arg, Ser, Thr,  
Trp or His

&lt;220&gt;

&lt;223&gt; Xaa at position 98 could be either Gly or Pro

&lt;220&gt;

<223> Xaa at position 99 could be either Ser, Thr, Ala  
or Leu

&lt;220&gt;

<223> Xaa at position 100 could be either Arg, Ser, Met,  
Thr or Leu

&lt;220&gt;

<223> Xaa at position 101 could be either Val, Ile, Thr,  
Met or Leu

&lt;220&gt;

&lt;223&gt; Xaa at position 32 could be either Asn, Gly or Tyr

&lt;220&gt;

&lt;223&gt; Xaa at position 33 could be either Thr or Asp

&lt;220&gt;

&lt;223&gt; Xaa at position 53 could be either Asp or Ser

&lt;400&gt; 8

Xaa Xaa Val Leu Thr Gln Pro Pro Ser Val Ser Gly Xaa Pro Gly Gln  
1 5 10 15Arg Val Thr Ile Ser Cys Xaa Gly Xaa Xaa Ser Asn Ile Xaa Xaa Xaa  
20 25 30Xaa Val Xaa Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu  
35 40 45Ile Tyr Xaa Asn Xaa Xaa Arg Pro Ser Gly Val Pro Asp Arg Phe Ser  
50 55 60Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Xaa Gln  
65 70 75 80Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Xaa Tyr Xaa Xaa Xaa Xaa  
85 90 95Xaa Xaa Xaa Xaa Xaa Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly  
100 105 110

&lt;210&gt; 9

- 6 -

<211> 6  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <223> Xaa at position 2 could be either Gly, Val, Cys or His

<220>  
 <223> Xaa at position 3 could be either Ser or Thr

<220>  
 <223> Xaa at position 4 could be either His, Thr, Val, Arg, or Ile

<220>  
 <223> Xaa at position 5 could be either Asp or Ser

<220>  
 <223> Xaa at position 6 could be either Asn, Lys, Ala, Thr, Ser, Phe, Trp, or His

<400> 9  
 His Xaa Xaa Xaa Xaa Xaa  
           1                  5

<210> 10  
 <211> 12  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <223> Xaa at position 4 could be either Asp or Ser

<220>  
 <223> Xaa at position 5 represents any amino acid

<220>  
 <223> Xaa at position 6 could be either Gly, Asp, Gln, Leu, Phe, Arg, His, Asn or Tyr

<400> 10  
 Gln Ser Tyr Xaa Xaa Xaa Thr His Pro Ala Leu Leu  
           1                  5                  10

<210> 11  
 <211> 17  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <223> Xaa at position 1 could be either Phe, Thr or Tyr

<220>  
 <223> Xaa at position 3 could be either Arg or Ala

<220>  
 <223> Xaa at position 5 could be either Asp, Ser, Glu or Ala

<220>  
 <223> Xaa at position 6 could be either Gly or Arg

<220>  
 <223> Xaa at position 8 represents any amino acid

- 7 -

&lt;220&gt;

&lt;223&gt; Xaa at position 10 could be either Tyr or Glu

&lt;400&gt; 11

Xaa	Ile	Xaa	Tyr	Xaa	Xaa	Ser	Xaa	Lys	Xaa	Tyr	Ala	Asp	Ser	Val	Lys
1				5					10					15	

Gly

&lt;210&gt; 12

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Xaa at position 1 could be either Gly, Tyr, Ser, Thr, Asn or Gln

&lt;400&gt; 12

Xaa	Asn	Asp	Gln	Arg	Pro	Ser
1				5		

&lt;210&gt; 13

&lt;211&gt; 9

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Xaa at position 4 and 5 represents any amino acid

&lt;220&gt;

&lt;223&gt; Xaa at position 6 could be either Tyr or His

&lt;220&gt;

&lt;223&gt; Xaa at position 7 could be either Gly, Met, Ala, Asn or Ser

&lt;400&gt; 13

Phe	Thr	Phe	Xaa	Xaa	Xaa	Xaa	Met	His
1						5		

&lt;210&gt; 14

&lt;211&gt; 13

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;220&gt;

&lt;223&gt; Xaa at position 9 could be either Ser, Cys, Arg, Asn, Asp or Thr

&lt;220&gt;

&lt;223&gt; Xaa at position 10 could be either Asn, Met or Ile

&lt;220&gt;

&lt;223&gt; Xaa at position 11 could be either Thr, Tyr, Asp, His, Lys or Pro

&lt;400&gt; 14

Ser	Gly	Gly	Arg	Ser	Asn	Ile	Gly	Xaa	Xaa	Xaa	Val	Lys
1					5					10		

- 8 -

<210> 15  
 <211> 114  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <223> Xaa at position 30 could be Ser or Glu

<220>  
 <223> Xaa at position 83 could be Lys or Asn

<220>  
 <223> Xaa at position 5 could be either Gln or Glu

<400> 15  
 Gln Val Gln Val Xaa Ser Gly Gly Gly Val Val Gln Pro Gly Arg Ser  
     1                    5                    10                    15  
 Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Xaa Tyr Gly  
             20                    25                    30  
 Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ala  
             35                    40                    45  
 Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys  
     50                    55                    60  
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu  
     65                    70                    75                    80  
 Gln Met Xaa Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Lys  
             85                    90                    95  
 Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr Val  
             100                    105                    110  
 Ser Ser

<210> 16  
 <211> 112  
 <212> PRT  
 <213> Homo sapiens

<220>  
 <223> Xaa at position 1 could be either Ser or Gln

<220>  
 <223> Xaa at position 2 could be Tyr or Ser

<220>  
 <223> Xaa at position 13 could be either Thr or Ala

<220>  
 <223> Xaa at position 25 could be either Gly or Ser

<220>  
 <223> Xaa at position 51 and 95 could be either Gly or Tyr

<220>  
 <223> Xaa at position 79 could be either Val or Leu

<400> 16

- 9 -

Xaa Xaa Val Leu Thr Gln Pro Pro Ser Val Ser Gly Xaa Pro Gly Gln  
 1 5 10 15  
 Arg Val Thr Ile Ser Cys Ser Gly Xaa Arg Ser Asn Ile Gly Ser Asn  
 20 25 30  
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu  
 35 40 45  
 Ile Tyr Xaa Asn Asp Gln Arg Pro Ser Gly Val Pro Asp Arg Phe Ser  
 50 55 60  
 Gly Ser Lys Ser Gly Thr Ser Ala Ser Leu Ala Ile Thr Gly Xaa Gln  
 65 70 75 80  
 Ala Glu Asp Glu Ala Asp Tyr Tyr Cys Gln Ser Tyr Asp Arg Xaa Thr  
 85 90 95  
 His Pro Ala Leu Leu Phe Gly Thr Gly Thr Lys Val Thr Val Leu Gly  
 100 105 110

<210> 17  
 <211> 6  
 <212> PRT  
 <213> Homo sapiens

<400> 17  
 His Gly Ser His Asp Asn  
 1 5

<210> 18  
 <211> 12  
 <212> PRT  
 <213> Homo sapiens

<400> 18  
 Gln Ser Tyr Asp Arg Gly Thr His Pro Ala Leu Leu  
 1 5 10

<210> 19  
 <211> 17  
 <212> PRT  
 <213> Homo sapiens

<400> 19  
 Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val Lys  
 1 5 10 15  
 Gly

<210> 20  
 <211> 7  
 <212> PRT  
 <213> Homo sapiens

<400> 20  
 Gly Asn Asp Gln Arg Pro Ser  
 1 5

- 10 -

<210> 21  
 <211> 9  
 <212> PRT  
 <213> Homo sapiens

<400> 21  
 Phe Thr Phe Ser Ser Tyr Gly Met His  
 1 5

<210> 22  
 <211> 13  
 <212> PRT  
 <213> Homo sapiens

<400> 22  
 Ser Gly Gly Arg Ser Asn Ile Gly Ser Asn Thr Val Lys  
 1 5 10

<210> 23  
 <211> 115  
 <212> PRT  
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<400> 23  
 Gln Val Gln Leu Val Gln Ser Gly Gly Gly Val Val Gln Pro Gly Arg  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr  
 20 25 30  
 Gly Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ala Phe Ile Arg Tyr Asp Gly Ser Asn Lys Tyr Tyr Ala Asp Ser Val  
 50 55 60  
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr  
 65 70 75 80  
 Leu Gln Met Lys Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys  
 85 90 95  
 Lys Thr His Gly Ser His Asp Asn Trp Gly Gln Gly Thr Met Val Thr  
 100 105 110  
 Val Ser Ser  
 115

<210> 24  
 <211> 112  
 <212> PRT  
 <213> Homo sapiens

<400> 24  
 Ser Tyr Val Leu Thr Gln Pro Pro Ser Val Ser Gly Thr Pro Gly Gln  
 1 5 10 15  
 Arg Val, Thr Ile Ser Cys Ser Gly Gly Arg Ser Trp Ile Gly Ser Asn  
 20 25 30  
 Thr Val Lys Trp Tyr Gln Gln Leu Pro Gly Thr Ala Pro Lys Leu Leu

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